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AZERBAIJAN NATIONAL ACADEMY OF SCIENCES

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PROFESSOR AHLIMAN AMIRASLANOV WHO HAS CONTRIBUTED TO MODERN MEDICINE

The present paper dedicated to active member of the Azerbaijan National Academy of Sciences, foreign member of the Russian Academy of Sciences, full member of the Russian Academy of Nature Sciences and Polish Medical Sciences, Academician-Secretary of the Department of Biological and Medical Sciences of the Azerbaijan National Academy of Sciences (ANAS), Editor-in-Chief of the Journal "Proceedings of ANAS (Biological and Medical Sciences)", Member of the Milli Majlis of the Republic of Azerbaijan and Chairman of the Health Committee, doctor of medical sciences, professor Ahliman Amiraslanov.



Medical science has also deep roots in Azerbaijan, which has ancient and rich culture and scientific traditions. Physical-biological health and moral-mental perfection of human being are presented in unity with each other in the majority of Azerbaijani spiritual culture patterns belong both to ancient and medieval era. Profession of a doctor is raised to the level of sacral missions rather than ordinary occupation in Middle Ages: as it is reflected in the works of our classics, the sacred duty of the doctors is to provide the physical-biological and mental health service, which is the essential condition of human inner-spiritual perfection. In the history of our national culture, we are observing this trend related to medicine in the twentieth century too. The tradition of approaching these classic traditions in the face of Mirasadulla Mirgasimov, Mustafa bey Topchubashov, Aziz Aliyev, later Zarifa Aliyeva, Jamil Aliyev and a great many per-

sonalities, lived in the most classic times of the Soviet epoch, was traditionally treated as a sacred mission rather than ordinary profession. These prominent scientists contributed greatly to medical science and our national health by combining the rich traditions of Azerbaijan's medical science with modern scientific knowledge and achievements. Contemporary Azerbaijan medical science, which is based on rich scientific traditions, incorporates the name and signature of many prominent scientists and whole-hearted physicians who have healed many people. Ahliman Amiraslanov has a special place and position among these prominent names and signatures. Ahliman Amiraslanov is a well-known person with exceptional services in the development of Azerbaijan medical science on the 20th century, who was able to bring together in his activities the wide scientific, scientific-organizational, pedagogical, and social field of activities and distinguishing always by his real citizen-intellectual position.

Active member of the Azerbaijan National Academy of Sciences, foreign member of the Russian Academy of Sciences, active member of the Russian Academy of Natural Sciences and Polish Academy of Medical Sciences, Honoured Scientist, laureate of the USSR State Prize, member of the Azerbaijan National Assembly (Milli Majlis) of the Republic of Azerbaijan and Chairman of the Health Committee, Academician-Secretary of the Department of Biological and Medical Sciences of ANAS, doctor of medical sciences, professor Ahliman Tapdig Amiraslanov was born on November 17, 1947 in Zod village of Basarkechar district, Goycha region of Western Azerbaijan in an intelligent family. His father Tapdiq Mukhtar Amiraslanov (1918-1976) worked as a director of secondary school, party leader and head of educational department in Basarkechar district and dedicated all his strength and abilities to the development of the native land, enlightenment of the motherland, and deserved the title of "Honoured teacher" for his long-term complete pedagogical activity. Her mother, Khanum Ilyas Aliyeva (1922-2006) devoted her life to the education and care of her children.

In 1965 A.T.Amiraslanov graduated from secondary school with gold medal and entered the Medical-Prophylaxis Faculty of Azerbaijan Medical Institute named after N. Narimanov in 1965. While studying at the institute young Ahliman was distinguished by inborn talent, high erudition, and displayed a keen interest in oncology field and, while still a student, he began his labor activity as a male nurse at the Baku Clinical Oncology Hospital in 1969. After graduating with honour diploma from the Institute, he worked as a surgical oncologist and head of department at the surgical department of the Baku City Oncology Hospital in 1971-74. His first teacher on oncology was a prominent scientist, Professor Arif Abbasov.

As known, the 60-70s of the last century was a period of high socioeconomic and spiritual-cultural rise in the life of the Azerbaijani people. Azerbaijan become one of developed republics of the USSR in a short period thanks to national leader Heydar Aliyev who came to power in Azerbaijan and carried out reforms in the country. In those years, one of the important directions of Heydar Aliyev's reforms was organization of the training of highly qualified personnel abroad. In those years, Ahliman Amiraslanov decided to go to Moscow to enrich his oncological knowledge in favorable conditions created by Heydar Aliyev.

A.T.Amiraslanov entered the postgraduate course at Moscow All-Union Scientific Center of Oncology (AUSCO) under the Academy of Medical Sciences of the USSR (Moscow) in 1974. In 1977 he completed the postgraduate study and defended his PhD thesis on "Rehabilitation of patients after amputation of lower extremities due to malignant tumors" and was honored with the scientific degree of the candidate of medical sciences. Typically, during the Soviet Union, graduates from postgraduate course in Moscow's educational and scientific centers had to defend their dissertation and return to their republics immediately after receiving a scientific degree. However, after graduating a post graduate course, at the suggestion of the Director General of the AUSCO academician N.N. Blokhin and A.T.Amiraslanov's scientific supervisor academician N.N. Trapeznikov, the young scientist was kept in the world-known scientific research center. Thus, A.T.Amiraslanov had worked as a junior scientific worker at the General Oncology Department of the USSR AMS in Moscow since 1977, since 1981 he had worked as a researcher in that department, and since 1984 as a researcher. During this period, numerous complicated surgical operations, including Rotational Plastics, were performed skillfully by Azerbaijani scientist A.T.Amiraslanov, which had been performed only in several world-renowned clinics of the USSR, and hopeful

results were achieved. The young scientist continued his scientific research successfully and in 1984 he was awarded with Doctorate degree in medical sciences at the age of 36 defending his thesis on "Comprehensive treatment methods for patients with osteogenic sarcoma".

The scientist, who has been widely commented on "all the subtleties of bone sarcoma" with his scientific works in the world press and distinguished by his remarkable reports and speeches at international scientific events, is well-known and respected not only in different republics of the Soviet Union, but also throughout the world.

A.T.Amiraslanov was awarded with various awards for his effective scientific research carried out by the leadership of the USSR Academy of Medical Sciences (AUSCO) in Moscow in 1979-1985. Over these years, at the Department of General Oncology, the prominent scientist A.T.Amiraslanov was charged for the theme "Improving the treatment methods of bone tumors" and he was able to cope with this task.

The scientist has consistently applied several scientific studies to health practice. Among them, rehabilitation treatment methods for treatment of tumors during musculoskeletal system oncology have occupied the main place.

Azerbaijani scientist led the All-Union School on the "Rehabilitation and Treatment of Patients with stand-motion apparatus tumors" in 70-80s of the XX century and was repeatedly awarded with gold medals of the USSR People's Economy Achievements Exhibition.

A.T.Amiraslanov's elaboration "Comprehensive treatment of osteogenic sarcoma in children, adolescents and the adults" was included in the All-Union perspective plan of the application of significant achievements of medical science in the field of health practice by the Order of the Minister of Health of USSR Minister dated October 3, 1983.

The productive scientific activity of A.T.Amiraslanov was reflected in the monograph "Bone sarcomas" in 1985, and this original publication was awarded Prize and medal of the Academy of Medical Sciences of the USSR named after academician N.N. Petrov, which is presented once in 5 years. The scientist, who gained great reputation in the international arena, was awarded the USSR State Award in Science and Technology in 1986 for a series of scientific and technological works on the "Elaboration of methods for the treatment of oncological patients and their application in clinical practice".

Even N.N. Trapeznikov, a prominent world-famed expert, notes that the great majority of the results of the scientific research carried out by Ahliman Amiraslanov in Moscow are the first sig-

nificant scientific achievements in the USSR, and it either advance the scientific results of foreign scientists in respective fields, or complete them.

A.T.Amiraslanov was assigned as a leading scientific worker at the Department of General Oncology of AUSCO in 1987, and in 1989 he was elected as a professor and then worked in this post until 1992.

Professor Amiraslanov was involved in the field of therapeutic practice, in addition to important scientific research and organizational work at the Center of Oncology of the Russian Academy of Medical Sciences. He was a member of the Specialized Board on Oncology, as well as the member of Scientific Board of the Oncological Center of the Academy of Medical Sciences of the USSR and member of the permanent commission on hospitalization organized at the AUSCO. In 1988, he worked actively in the composition of the International Organizing Committee of the Oncology School titled "Treatment of Bone sarcomas" at USSR AMS.

During his work in Moscow, the scientist supervised directly the research work for 12 PhD theses and 5 doctor's degree theses in medical sciences.

Professor A.T. Amiraslanov was awarded the honorary title of "Honored Scientist" in 1991 for the preparation of highly qualified medical staff, as well as for the development of medical science and health.

The end of the 80s of the last century, the beginning of the 1990s was very difficult and hard for Azerbaijan. As in many other fields, many specialists in the field of medical science left Azerbaijan and went to other countries in these years. In the period when Azerbaijan experienced with occupation of our lands, Ahliman Amiraslanov refused from the post suggested to him as Director of Clinical Oncological Institute and decided to arrive in Baku and to be a supporter with his experience as professional doctor, scientist and with pedagogical activity in the hard years of Azerbaijan. It should be noted that A.T.Amiraslanov, as usual, gained the respect and love of the staff and the people with his kindness, intelligence, disinterested doctor and professional service. The scientist was glad to serve his own people and to be with the people in hard times. It is a gratifying fact that during the past years in Moscow, these features did not leave Ahliman Amiraslanov for a moment. He has never changed the rhythm of the occupation of doctor, regardless of what he works in public affairs, in state position, has tried to engage in daily work, hope and healing for his patient, and considered a child's duty to serve his homeland. Therefore, the people recognize and love him not only as a world-known scientist, skilful organizer, competent educator and

health worker, but also as an educated, careful, and sociable doctor.

Ahliman Amiraslanov was elected as the rector of Azerbaijan Medical University in 1992 and worked about 24 years. In 1993, he was elected as a director of the Oncology Department of the Azerbaijan Medical University through vacancy announcement, and he is also a director of the Oncological Clinic of AMU. Along with the great success, he has gained in his work as a scientist and doctor, his rich experience in education and in organization of scientific activities have started to yield good results since his early days as the rector of AMU. Under A.T.Amiraslanov's direct guidance the education system in AMU has adapted to international standards, the University has turned world-class science, education and practice center thanks to numerous reforms he has undertaken in. It should be noted that, in particular, during the rectorship, the organization of training of doctors and specialists in the country and abroad, Bologna system that AMU joined, the organization of AMU's joining the Bologna system, transition to a credit system of education, reforms, such as the establishment of oncology, dentistry, educational-therapeutic and educational-surgical clinics at the University, the expansion of international relations and other reforms like this can be considered the most prominent events in the history of medicine and science of medicine.

As a result of the scientific, pedagogical and organizational activities of A.T.Amiraslanov's personal selflessness on the areas of international relations, in 1996, among many republics of the USSR, including the Caucasian republics, only Azerbaijan Medical University was included in the International Register of American and European Education Association published in London, and in 1998 was included in the Union of High Schools of the Black Sea Basin countries and was selected as a full member of the International Association of High Schools in 2000, the European Association of Universities in 2002, and the Turkish University of Union in 2011, as a whole, all these have become an important factor in the development of the Azerbaijani medical education system internationally, as well as the mutual recognition of diplomas.

The introduction of the credit system in education and Bologna process had been launched at the Faculty of Pharmaceutics of AMU in 2000 and in 2006, in other faculties, the Electronic Test Exam Center was established, semester and final exams were started with computers. A skill laboratory for students and residents was organized for the first time in the history of the university. A.T.Amiraslanov was a great contributor in the creation of the military medicine faculty under AMU.

One of the most important services of academician A.T.Amiraslanov has been the creation of bases for clinical training and training of specialists in the University, as in foreign countries, for the future of medical science. As it is known, in 2000, as in other post-Soviet republics, medical education and training of specialists in our country did not meet international standards applied in leading world educational centers, clinical studies were carried out in state hospitals due to the lack of university hospitals.

Owing to intensive organizational activities initiated by the University administration for the first time, the training of highly qualified personnel for the republic started in foreign countries, especially in Turkey and in European countries. Both employees of University and students were sent abroad, and they were offered facilities for specialization. These cadres returning to the country played an exceptional role in establishing modern medicine in Azerbaijan. Stomatology clinic established in 2002 with the support of the Great Leader Heydar Aliyev, Oncological clinic, established in 2007, training and therapeutic clinics established in 2010 with the support of the President of the Republic of Azerbaijan Ilham Aliyev and Training and Surgery Clinics opened in 2013 formed foundation of the modernization and further development of Azerbaijani medicine. Simulation centers created in these clinics today play an exceptional role for students and residents in acquiring clinical and practical skills. A.T.Amiraslanov had worked hard for many years to organize an international medical training system in our country, and finally, in 2011 the residency education began to be implemented in Azerbaijan. During his rectorship, several educational buildings were built, lecture-halls were modernized, wide use of electronic means in training began, international relations expanded, contracts and exchanges with world leading scientific and medical centers were implemented. Amiraslanov has provided great services in the development of medical science both in the Medical University and in our country. During his rectorship, more than 1,000 scientists with PhD degree; more than 100 scientists with Doctor's degree were trained, about 300 monographs, more than 400 textbooks and manuals, more than 20,000 scientific works were published by university staff, more than 100 copyrighted works were received, more than 200 scientific-practical conferences were organized.

AMU has collaborated with about 10 international projects and grants such as TEMPUS, ERASMUS MUNDUS, ERASMUS Plus programs of the European Union, the MEVLANA exchange program of the Turkish Republic, more than 500 students, about 200 members of professors-teacher

staff have implemented joint research on exchange and global issues. As a result of A.T.Amiraslanov's direct efforts, scientific research projects and grants have been jointly implemented with 135 universities (Hospital of Koln University, Hannover Medical School, Berlin Humboldt University, University of Glasgow, University of Duisburg (Essen), Sofia Medical University, Tbilisi State University, Tehran Medical Science University, La Sapienza University, Polish Medical Academy, Almaty State Institute of Doctors Improvement, Bratislava University, Marmara University, Istanbul University, Ankara University, Hajettepe University, Inonu University, First Moscow State University named after I.M. Sechenov and others) 33 countries (Germany, Belarus, Bulgaria, Great Britain, Estonia, France, Georgia, Iran, Spain, Sweden, Italy, Israel, Korea, Latvia, Lithuania, Netherlands, Norway, Pakistan, Poland, Portugal, Kazakhstan, Russia, Slovakia, Turkey, Ukraine, Greece etc.).

Amiraslanov personally played an active role in organization of international conferences in Azerbaijan and abroad and made every effort to represent our country at the highest level.

He chaired section of medicine and biology of the I-IV Baku Humanitarian Forum, held in Baku being one of the largest scientific and political events in the world, and organized discussion of global scientific issues as "Biotechnology and Ethics Problems" (2011), "Technology and molecular biology in modern science" (2012), "Molecular Biology and Biotechnology Achievements: From Theory to Practice" (2013), "Molecular Biology and Biotechnology in the XXI century: Theory, Practice, Perspectives" (2014) with the participation of world famous experts.

Amiraslanov organized lectures inviting world-influential scientists including Nobel Prize winners (Professor Kurt Wutrix, Professor Thomas Kristian Zudhof, Professor Ada Yonath, Professor Ervin Neher, Rudolf Martin Tsinkernagel, Professor Ariye Warsaw, Professor Keri Benks Mullis, Nikolay Zefirov) to the University and enabled them to share their scientific and practical knowledge with professors-teachers and students. During his rectorship, many well-known scientists from around the world were awarded the "Honorary Doctorate" of the AMU (Ihsan Dogramaji (Turkey), Harmen van Lessen (Germany), Javad Hayat (Iran), Mehmet Haberal (Turkey), Anvar Hasanoglu (Turkey), Andreas Ebert (Germany), Mikhail Ivanovich Davydov (Russia) and "Honorary Guest" (Murat Tuncer (Turkey), Albert Somingen (Germany), Roland Hetzer Karsten Perka (Germany). Employees of the Azerbaijan Medical University made over 1500 visits related to their research works to more than 90 countries in 1992-2016.

An important part of the research and scientific-organizational activity of academician A.T.Amiraslanov is related to the Azerbaijan National Academy of Sciences.

He was elected an active member of ANAS in 2001, he has been a member of the Presidium of ANAS and Academician-Secretary of the Department of Biological and Medical Sciences (DBMS) since 2007. He plays a leading role in defining the main directions of biology and medical sciences in our country and in the organization and modernization of scientific works. He leads and provides all-round support to conduction of studies based on the modern methods, to intensive development of international relations, establishment of new scientific institutions, continuous improvement of the facilities in relevant fields. Academician Ahliman Amiraslanov is a member of the Republican Scientific Research Coordination Board. He provides overall supervision of the activities of scientific boards established on Biological Problems, Medical Problems, Genetics and Selection Problems of Agricultural Plants and Animals, Land and Water Resources Management and Environmental Protection Problems that operate in the structure of the Board.

During his activity at Academy, priority directions and research topics on biology and medicine in Azerbaijan have been identified and currently being successfully implemented. These include the study of biodiversity, soil and water resources with the latest technology, the development and application of fundamental bases of new molecular and cell biotechnology, the production of gene and extracts in plants, the creation of new plant varieties by using molecular and genome selectivity, human genetics and genomics, prenatal and neonatal diagnosis of hereditary diseases, preparation of pharmaceutical products and preparation of pharmaceutical preparations using plant expulsion systems, stem, cell technology, physiological and molecular-genetic therapy of longevity and neurodegenerative diseases, investigation of new methods of treatment with application of modern technologies and so on. Under the guidance of academician A.T.Amiraslanov, the institutes and staff of Department of Biology and Medical Sciences (DBMS) have been actively involved in the preparation and implementation of strategically important state programs, as "Reliable provision of population with foodstuffs", "Poverty reduction in Azerbaijan", "Alternative and Renewable Energy Sources", "Drugs and Struggle against Drugs Abuse", "Azerbaijani Youth in 2011-2015", "Azerbaijan 2020: Look into the Future", "Development of non-oil industry", "Non-infectious diseases", etc. and as well as important documents such as "National Strategy for Science Development", "National Strategy and Action Plan for

the Protection and Sustainable Use of Biodiversity in the Republic of Azerbaijan" and others. During his activity, substantial changes were made in the structure of the DBMS. The Institute of Molecular Biology and Biotechnologies was established under the department, Mardakan Dendrary has turned into the Institute of Dendrology of ANAS, Genetic Resources Institute, Institute of Soil Science and Agricultural Chemistry were included in the DBMS and the staff training on the Master's Degree started. In order to modernize the organization of scientific research works, websites of the research institutes included in DBMS were created and improved. The section of the "electronic database of dissertations" created on the website of the department plays an important role in the planning and coordination of the dissertation works in relevant fields. During his activity the following important scientific-organizational works were carried out in the Department under guidance of A.T.Amiraslanov: creation and upgrading the official website of the journal "Proceedings of ANAS (Biological and Medical Sciences)" and serious oversight of the articles published in the journal, the second edition of the "Red Book of the Republic of Azerbaijan" (2013), preparation of "National Atlas" (2014) should particularly be noted.

Real innovator of science organizer academician A.T.Amiraslanov, has started to make use of the principles of "modernity, multidisciplinary, multi-centrism and international relations in scientific researches" and has received more than 200 important scientific results in biology and medicine in the last 10 years, more than 80 patents have been obtained, nearly 140 books have been published, nearly 260 grant projects have been implemented, the number of articles published in repetitive journals has increased several times. Fruitful cooperative relationships have been established between research institutes of DBMS and a number of the world's leading research centers (UK, USA, Turkey, Germany, France, Switzerland, Italy, Canada, Japan, Russia, Ukraine, Belarus etc.) and scientists, joint-research work has been carried out and specialist exchanges have been undertaken. Opportunities for young researchers (including opportunities for participation in master's and doctoral studies in international training and research programs, prominent universities in developed countries) have been expanded, Master's degree studies in several institutes of the department has been organized at the highest level, admission to doctorate has increased.

The main direction of academician A.T.Amiraslanov's research work includes musculoskeletal system oncology soft tissues, malignant tumors of the breast and a number of internal organs, as well as the development and improvement of their effective

treatment methods, preventive measures and their successful application in practice. Scientific researches conducted under his supervision include reconstructive treatment (rehabilitation) of patients with surgical intervention for malignant tumors of the musculoskeletal system; Development of new methods for treating bone sarcoma tumors; prognosis of malignant tumors of bones; hormonal-methodological changes in malignant tumors of female genital organs and bones; elaboration of combinative complex treatment and modern methods for soft tissue sarcomas; epidemiology of some malignant tumors in Azerbaijan; early diagnostics of malignant tumors and the clinical significance of paracrine and immunohumoral factors in the prognosis; peculiarities of angiogenesis in various localized malignant tumors, and other topics are of priority.

Academician A.T.Amiraslanov is the author of a number of medical and biological technologies for the diagnosis, prophylaxis, treatment, rehabilitation of the oncological diseases and the application of obtained results in practice. Technically complicated surgical operations such as resection and endoprosthesis of the knee, hip-pelvis and humeral joints in malignant tumors of the hip, reed and humeral bones, iliac-stomach separation, amputation of the shoulder-blade pectoral are performed successfully in the Oncology clinic under his leadership.

The new methods of treatment offered by the scientist in the relevant fields have already been tested and applied successfully in practical healthcare and are widely used in various clinics. These methods accelerate the recovery of the illness, reduce the disability, and help patients return to active labor and life in a short time.

Academician A.T.Amiraslanov's scientific works have been widely spread in not only Azerbaijan and CIS, but also in other leading countries of the world, a solid foundation has been created for the beginning of a new phase in oncology science. He has repeatedly participated in an international congress, symposium, congresses and conferences held, in the United States, France, Japan, Italy, Sweden, Greece, Hungary, Bulgaria, Czech Republic, Germany, Turkey, Russia, Poland, Israel, Pakistan, Canada, Iran, Austria and in other countries and has successfully presented his achievements with his scientific reports in the field of oncology.

The prominent scientist, as the head of USSR delegation and now as the head of independent republic delegation, repeatedly was on a scientific mission and made a worthy contribution to the strengthening of scientific and pedagogical relations at the international level.

He has also been for a long time on a scientific mission at the Houston Medical Centers in the United States, including the M.D.Anderson Oncol-

ogy Clinic. Academician A.T.Amiraslanov, one of the world's most renowned oncologist-surgeons, has been representing Azerbaijan's medical science on a world scale with dignity by reading profound content and valuable lectures in many influential foreign clinics.

A.T.Amiraslanov as an experienced pedagogue has gained profound respect and great authority by delivering interesting and pithy lectures to the students of Central Qualification Improvement Institute in Moscow, graduates and ordinators of Scientific Board of the Center for Oncological center of the Academy of Medical Sciences of the USSR (AUSCO), as well as to the students, residents and listeners of Azerbaijan Medical University, in Baku.

Academician Ahliman Amiraslanov is the author of about 500 scientific works, including 18 monographs, 31 textbooks, training aids and methodical literatures, 9 inventions and rationalization proposals. The large majority of his works were published in many influential journals, collections and magazines of the world. He is also the author of dozens of scientific articles, writings dealing with the life and creativity of prominent personalities. Academician A.T.Amiraslanov's, first original textbook "Oncology", has made an invaluable contribution to the Azerbaijani students, and has become a handbook of doctors and post-graduate students. His book titled "Prognosis of malignant Bone Tumor", published in Ankara in 1995 and Monographs on "Oncological cachexia", published in 1999, also gained the sympathy of wide range of readers.

The book entitled "Cachexia in Patients with Stomach and Gullet Cancer" was published in Russian and English languages in Turkey, and was given to the use of world readers. In addition, in 1987 in New York, 1989 in Tokyo, articles on the valuable results of A.T.Amiraslanov's current studies on modern oncology were widely presented in a large section of each of the remarkable managers edited and by worldwide influential oncologist W.F.Enneking and T.Yamamura.

Academician A.T.Amiraslanov pays special attention to the issue of preparing scientific and pedagogical staff and works productively in this field, without reducing their demand for a moment and approach them with a great deal of care, attention and sensitivity. The followers of Ahliman Amiraslanov's scientific school work in various clinics, universities, research institutions not only in Azerbaijan, but also in foreign countries and continue his researches. Under the direct supervision of A.T.Amiraslanov, 12 persons defended their theses for doctor's degree and 43 persons for PhD in medical sciences and were honored with corresponding scientific degrees.

Academician Ahliman Amiraslanov has been deputy editor-in-chief of "Proceedings of ANAS (Biological and Medical Sciences)" for many years, and was elected the editor-in-chief of this journal in 2016. He is one of the founders of AMU's "Tabib" publishing house and has played an active role in the broad range of publishing activities.

Academician A.T.Amiraslanov, on the basis of scientific and medical activity was elected full member of the Russian Academy of Medical Science in 2001. He is a foreign member of the Russian Academy of Sciences, member of the Universal Orthopedic Traumatology and Oncology Association, as well as member of European Association for Reproductive Surgery, Oncology Society of Greece, Czech Republic, Hungary, member of the American Society for Clinical Oncology, the full member of the World Medical Academy, the European Medical Oncology Society, the Russian Academy of Natural Sciences, and the Polish Academy of Medical Sciences. He is also honorary doctor of Gazi University of Turkey.

A.T.Amiraslanov was awarded with the honorary title "Scientist of 1997", according to a survey conducted in January, 1998. His name was included in the main part of the "2000 outstanding personality of the twentieth century" journal, published in Cambridge for his valuable contributions to world medical science. In 1998, he was awarded with the "Golden Statue" of the American Biographical Institute and in 1999 he was awarded with the Albert Schweitzer Award of the Polish Academy of Medical Sciences and the Grand Gold Medal in the same year in the field of medical science, for the development of medical education system, as well according to the high results gained for wide application of achievements in the field of oncology to health practice.

For achievements in the field of medical science, education, health and development of biological sciences in Azerbaijan, scientist was highly appreciated by the government and public. He was awarded the Order of "Shokhrat" (Glory) of the Republic of Azerbaijan in 2000. Academician A.T.Amiraslanov was elected Deputy Chairman of Azerbaijan National Committee "Bioethics, Ethics of Scientific Knowledge and Technology" for UNESCO in 1999, member of the Presidium of UNESCO Committee in 2006, and a Chairman of Committee on "Bioethics, Ethics of Scientific Knowledge and Technology" on June 29, 2016. He is an expert of UNESCO.

Academician A.T.Amiraslanov was awarded with the "Blokhin Gold Medal" of Academy of Medical Sciences of Russian Federation in 2007 and the international award "The Intellect of the

Nation" in 2009 within the framework of Russia's "Leaders of the XXI Century" program. He was awarded the honorary title of "Honored Scientist of the Republic of Dagestan of RF" by decree of the President of the Republic of Dagestan for contribution to the development of scientific cooperation between the Republic of Azerbaijan and the Republic of Dagestan.

According to the order of the President of Azerbaijan, the name of academician A.T.Amiraslanov was included in the list of the Board of Trustees of the Science Development Fund under the President of the Azerbaijan Republic (2010, 2013, 2017), the Board of Trustees of the Knowledge Fund under the President of the Azerbaijan Republic (2014), the State Awards Committee for Science, Technology, Architecture, Culture and Literature (2013), as well as in the list of members of the Editorial Board for preparation of the "National Atlas of the Republic of Azerbaijan". He was elected full member of the European Medical Oncology Society in 2011 and a member of the Coordination Board of World Azerbaijanis at the III World Congress of World Azerbaijanis.

Academician Ahliman Amiraslanov was elected as the deputy of the Supreme Soviet of the Azerbaijan SSR (Deputy) during the 12th convocation in 1990. During this period, he visited a number of foreign countries in the composition of state-level representations, took part in actively discussion of political issues defending Azerbaijan's interests, territorial integrity, protection of our independence and our statehood. He also acted efficiently as a member of the Inter-Parliamentary Assembly Council of the member States of the Commonwealth of Independent States. He is a Chairman of the Commission on Ecology and Natural Resources of the Parliamentary Assembly of Turkish-Speaking Countries (TURKPA) and the head of the Azerbaijan-Turkey Interparliamentary Friendship Group.

The activity of academician A.T.Amiraslanov as a Deputy of the Milli Majlis of the Republic of Azerbaijan in IV convocation (2010) and V convocation (2015) covered wide range of areas and served the interests of both those who voted for him and the people of Azerbaijan in general. His election as a chairman of the Milli Majlis's Health Committee in 2015 was a manifestation of the value given to his scientific-organizational and sociopolitical activities. As a result of Ahliman Amiraslanov's high organizational capacity, the Health Committee was formed shortly, its structures were established and a serious legislative activity was launched here.

Over the past period, the Health Committee held 17 meetings until September 2017, 31 bills

were discussed involving public representatives and specialists, more than 700 appeals of citizens were considered carefully, 538 letters were sent to the various addresses for the purpose of taking the measure on behalf of the Chairman of the Committee. The relations with international healthcare organizations have been significantly strengthened. In a short time, discussion of laws of the Republic of Azerbaijan on “Sanitary-Epidemiological Well-being”, “On Population Health Care”, “Psychiatric Assistance”, “State Care for Diabetes Mellitus”, “On Tuberculosis in Azerbaijan”, “State care to patients with hemophiliac and thalassemia hereditary diseases”, “Fight against illness caused by human immunodeficiency virus”, “On medical insurance”, “Amendments to the Code of Administrative Offenses of the Republic of Azerbaijan”, “On medicinal products”, “On food products”, “On advertising”, “On human's body transplantation and / or tissue transplantation” and so on and adoption of the amendments by Milli Majlis made to these laws under his leadership should particularly be mentioned. The amendments made at the initiative of Ahliman Amiraslanov to the law on “The list of items that are not permitted to be civilized (withdrawn from the civilian circulation)” and on “The list of things may belong to certain participants of civil circulation which are subject to certain permits (civilian circulation limited items)”, eliminate the difficulties associated with importing the iris to Azerbaijan and creating bank of the iris in our country.

Draft laws on “Approval of the Framework Agreement between the Republic of Azerbaijan and the Global Fund to Fight AIDS, Tuberculosis and Malaria”, on “Psychological Assistance”, “On

Compulsory State Insurance Rules for Virological Service” have also been prepared under academician's guidance and submitted to the Milli Mejlis. Academician Ahliman Amiraslanov's activity as the head of the Parliamentary working group on inter-parliamentary relations with Turkey is also estimable.

Round table discussions on “Strengthening Promotion of Maternal Health and Family Rights in Azerbaijan in the Framework of National Legislation” organized by the Health Committee together with the European Parliamentary Forum and the UN Population Fund in 2016 was held under chairmanship of A.T.Amiraslanov in 2016.

A conference on “Involvement of Parliaments of the Eastern Partnership in Promoting Women's Health and Gender Equality for Sustainable Development” was held in 2017 Milli Mejlis within the Euronest Parliamentary Assembly with the participation of academician A.T.Amiraslanov.

By the order of the President of the Republic of Azerbaijan on November 16, 2017, A.T.Amiraslanov was awarded the Order "Sharaf" (Honor). At the same time by the decision of the Presidium of ANAS he was awarded The Certificate of Honor.

Professor Ahliman Amiraslanov, who spent his whole life as a service of the people, healed people, and gave his life to the development of our science and health, today acts tirelessly with youth energy in scientific, scientific-organizational, pedagogical, medicinal and public spheres. As in all stages of the life of the scientist, today this activity is worthy of accomplishing his exceptional mission in the development of science, healthcare and education in Azerbaijan.

Molecular Analysis of Resistance Genes to Brown Rust *Lr9*, *Lr19*, *Lr34*, *Lr35* and Yellow Rust *Yr9*, *Yr18* in Azerbaijan Wheat Germplasm

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Rust is the most devastating fungal disease causing significant losses of grain production. Growing rust resistant varieties is the most sustainable, cost-effective and environmentally friendly approach for controlling rust diseases. To date, 81 brown and 53 yellow rust resistance genes have been detected in bread and durum wheat genotypes and their wild species using different molecular methods. The primary goal of the research was to establish the presence of *Lr* and *Yr* genes in wheat samples collected in Gene Pool of the Research Institute of Crop Husbandry (Baku, Azerbaijan). Four *Lr* genes, *Lr9*, *Lr19*, *Lr34*, *Lr35* and two *Yr* genes, *Yr9* and *Yr18* were analysed using various molecular markers. 1100 bp specific fragments in PCR profiles indicate the presence of *Lr9* gene on 6B chromosome of 60% of the studied 78 genotypes. Positive results were obtained in 45 genotypes for identification of *Lr19* gene using SCAR markers SCS123 and SCS253. Allelic state of *Lr34* gene was studied using *Lr34/csLV34a* and *Lr34/csLV34b* markers. Molecular analysis showed the presence of allele *a* of *Lr34* in 21 and allele *b* in 9 genotypes. Two genotypes were found to carry both alleles of *Lr34*. 54% of the studied wheat genotypes had no allele of *Lr34* gene. Fragments characteristic of *Lr35* gene were not visualized in PCR profiles of 61 genotypes. Fragments of 250 bp diagnostic for *Yr18* gene were visualized in electrophoretic profiles of 40 genotypes. 150 bp fragments characteristic of *Yr9* gene were amplified in all genotypes with the exception of four samples. These results will serve as a base for plant breeders to develop durable rust-resistant wheat varieties and to control wheat leaf rust diseases in Azerbaijan.

Keywords: Wheat, brown rust, yellow rust, *Lr9*, *Lr19*, *Lr34*, *Lr35*, *Yr9*, *Yr18*, PCR, STS, SSR, SCAR

INTRODUCTION

Rust diseases are the main factors decreasing productivity and quality of cereals all over the world (Aktar-Uz-Zaman et al., 2017), including Azerbaijan. Three rust diseases, namely, leaf or brown rust caused by *Puccinia triticina* Eriks, stem or black rust caused by *Puccinia graminis* f. sp. *Tritici* West, and stripe or yellow rust caused by *Puccinia striiformis* f. *tritici* Eriks, are the most economically significant and common diseases among global wheat cultivars. These diseases can cause the production loss up to 40-50% depending on the infection degree and duration. Food security is a strategy of our government and it is necessary to develop highly productive and resistant to biotic stress plants for providing the population with qualitative and ecologically pure food products. Despite the successes of the practical selection achieved on the issue of resistance, rust diseases still remain deleterious for cereals all over the world (Jighly et al., 2015; Periyannan et al., 2017). A resistant variety is considered as an important element in the integrated protective system of plants against diseases and pests. Therefore, the development of va-

rieties resistant to infections is more effective and ecologically safe method of struggling against rust diseases. Genotypes with a high genetic potential for the rust diseases resistance are of great practical importance. Thus, these genotypes are sources of the disease resistance genes and in the future they can be successfully used in molecular selection programs as parental forms. The permanent search for new donors, protected with genes, which are new for selection, and easily transfer properties at crossing is necessary for the development of resistant varieties (Riar et al., 2012; Abou-Elseoud et al., 2014; Hubbard et al., 2015).

Since the beginning of the XX century in various countries the investigations have been carried out to develop productive varieties, tolerant to adverse climatic conditions and diseases. Hybridological analysis revealed that genes for rust diseases resistance can function independently from each other or they can manifest complimentary or duplicate effects (Abdelbacki et al., 2013; Maccaferri et al., 2015). Moreover, an additional interaction, eliminating the effectiveness of the major gene, can occur between resistance and parental genes. In some cases additional resistance genes stimulate the

effectiveness of the major gene in varieties sensitive to diseases. Various genes can interact showing cumulative effect. The rust resistance genes are divided into two categories in the scientific literature: major genes or oligogenes and secondary or minor genes. The latter includes modifier genes, additional genes, non-specific resistance genes etc.

Currently, over 81 resistance genes against brown rust disease have been identified in wheat genotypes by various genetic and biochemical approaches (McIntosh et al., 2008; McIntosh et al., 2011, Aktar-Uz-Zaman et al., 2017). The majority of them are juvenile genes (expressing in youth period of the plant). *Lr12*, *Lr13*, *Lr21*, *Lr22*, *Lr34*, *Lr35*, *Lr37* are mature plant genes. The number of *Lr* genes effective against rust causatives is getting less every year (Ittu, 2000). Sexual hybridization and other processes occurred in pathogen cause the formation of virulent biotypes and forms, overcoming existing resistance. Therefore, a permanent search for new genes is necessary. Such an approach is important and actual for selection. Recently, using molecular biological methods DNA markers have been developed, which in combination with the respective resistance genes are powerful tools in the identification of genes (Cheikowski and Stepien, 2001).

Extensive investigations have been carried out on identification of resistance genes against wheat rust diseases and determination of their effectiveness in different geographical regions of the world (Mesterhazy et al., 2000, Abou-Elseoud et al., 2015, Imbaby et al., 2014). For example, using molecular markers and standard genetic methods *Lr3* gene 42%, *Lr26* - 28%, *Lr13* - 13%, *Lr37* - 9%, *Lr10* - 4%, *Lr1*, *Lr14a*, *Lr17b* - 1% were found in genotypes in Greece and Czechia. The gene *Lr37* appeared to be more effective against *P. triticina*, while the genes *Lr1*, *Lr10*, *Lr13* were effective only in combination with other genes. Australian wheat genotypes were shown to be protected from brown rust by the genes *Lr13*, *Lr24*, *Lr34*, *Lr37* and from yellow rust by the genes *Yr17* and *Yr18*. It is very interesting that the genes *Lr34* and *Yr18* are linked and they both are widely distributed in selection materials of CIMMYT, South and North America and China (Li et al., 2010). The gene *Lr13* has been providing durable protection in the Australian continent for more than 20 years (Huerta-Espino et al., 2011). As in Australia this gene is protecting more than a half of the varieties cultivated in England. *Lr26* (22%), *Lr37* (20%), *Lr10* (17%), *Lr17b* (*LrH*) (10%), *Lr1* (7%), *Lr3a* (6%) and *Lr20* (4%) are less frequent genes (Powell et al., 2013).

The genes *Lr23*, *Lr26*, *Lr34* and *Lr13* have been identified in Indian varieties. In Iraq mainly

the genes *Lr3*, *Lr10*, *Lr16*, *Lr17*, *Lr23*, *Lr26*, *Lr13* and *Lr1* and their combinations provide resistance.

Scientists from Russia and CIS countries also begin performing studies in this direction (Sibikeyev et al., 1996; Tirishkin, 2006; Karelov et al., 2001). The genes *Lr9*, *Lr19*, *Lr24* and *Lr38* were found to be effective against *P. triticina* population in the Russian territory.

To date 53 stripe rust resistance genes (*Yr1*–*Yr53*) and numerous temporarily designated genes have been reported in wheat (<http://wheat.pw.usda.gov/cgi-bin/graingenes>).

Most of these genes have been mapped on chromosomes and/or specific chromosomal regions, and many of them have been used in wheat breeding programs worldwide (Zhang et al., 2013). At first, eight resistance genes against yellow rust disease were detected and denoted as *Yr1*, *Yr2*, *Yr3* etc. Then the genes *Yr3* and *Yr4* were divided into other genes. Currently a lot of yellow rust resistance genes have been identified. Among these genes *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr16* are mature plant genes. Yellow rust resistance genes were detected mainly in bread wheat (*Triticum aestivum*) (McGrann et al., 2014; Basnet et al., 2014). However, most genes were transferred from other species and wild cereals by introgression. For example, the gene *Yr8* was transferred to bread wheat from *Aegilops comosa*, *Yr9* from rye, *Yr24* and *Yr28* from *Aegilops tauschii*, and *Yr26* from *Haynaldia villosa*. Until now a lot of useful alien genes have been transferred to wheat plants. But it is not possible to use all of them in the selection of commercial varieties, as alien chromosomal segments are not able to compensate losses of wheat native chromosomes or they have undesirable genes decreasing grain yield and quality (Powell et al., 2013; Luo et al., 2008).

During the last decade the number of named and mapped resistance genes in wheat increased pronouncedly (McIntosh et al., 1998; McIntosh et al., 2005; McIntosh et al., 2007). However, the wheat genome (17.3 pg per cell) belongs to the most numerous cultivated species and contains nearly 17000 Mbp per haploid nucleus. Because of the size and high percentage (over 90%) of non-coding sequences and A, B and D genomes with 7 homologous chromosomes it is difficult to perform molecular identification and cloning of wheat resistance genes. The average size of each (in total 42) hexaploid wheat chromosome is 800 Mbp. Physical distance between crossing-overs (=1 cM) varies from 0.3 to 3.0 Mbp (Feuillet et al., 1997). Wild relatives of wheat usually have one common genome, which is very useful for searching and mapping new resistance genes. Since wheat-related species carry different genomes (*Triticum* sp., ge-

nome B; *Aegilops speltoides*, genome S similar to B; *Triticum boeoticum*, genome A; and *Aegilops squarrosa*, genome D) they were and still are used as sources of resistance genes in plant breeding. A usual way of transferring the resistance genes is using wheat lines with translocation of a chromosome fragment carrying a wild species gene. This transfer was performed for the genes *Lr19*, *Lr24* and *Lr29* derived from *Agropyronelongatum* (Schachermayr et al., 1995, Prins et al., 1996). RFLP, RAPD, CAPS, SCAR, STS markers have been obtained using new DNA-based methods to identify individual resistance genes in wheat (Cheikowski and Stepień, 2001).

Revealing the genes by markers for detection of protein encoding sequences of nucleotide-binding sites and leucine-rich repeats (NBS/LRR) seems to be a perspective method for identification of the resistance genes. The mentioned sequences are available in some resistance genes and have been identified in various crops (tomato, potato, wheat, rice, flax) and also in model species such as *Arabidopsis* and *Nicotiana* (Leister et al., 1999, Salamini, 1999, Salman et al., 2000). Using RFLP probes from *Aegilops squarrosa* (*Triticum tauschii*) having resistance genes against cyst nematode of cereals, 29 loci that presumably are homologous to the analogs of the resistance genes were identified. These loci are identical to the amino-acid sequence of cyst nematode cre locus in *T. Tauschii* by 30-70%.

Using RFLP molecular markers *Lr1*, *Lr9*, *Lr10*, *Lr13*, *Lr19*, *Lr23*, *Lr24*, *Lr25*, *Lr27*, *Lr28*, *Lr29*, *Lr31*, *Lr34*, *Lr35*, *Lr37* and *Lr47*, which are the rust resistance genes of leaves were mapped on chromosomes. Despite the reliability of RFLP markers, they are very expensive and labor-intensive. DNA of high purity is required for these markers. Therefore, they are not suitable for the marker assisted selection. For practical purposes RFLP markers related to a corresponding resistance gene were converted to specific PVR markers-STs and CAPS. RAPD markers can be converted to SCAR ones (Cheikowski and Stepień, 2001).

Until now STS or SCAR and CAPS markers for the genes *Lr1*, *Lr9*, *Lr10*, *Lr24*, *Lr28*, *Lr35*, *Lr37* and *L47* have been reported. The enzymatic marker (endopeptidase Ep-D1c) for the gene *Lr19* was also developed and used. (Winzeler et al., 1995). Furthermore, microsatellite markers (simple sequence repeats-SSR) for the resistance genes *Lr3bg* and *Lr18* were also developed.

Moreover, molecular markers can be used in the pyramiding of various resistance genes in classical breeding process to achieve durable resistance. The durable resistance in one variety can be achieved by combination of several genes rather than by a single gene, partly encoding resistance

(Cheikowski and Stepień, 2001). The development of PCR-based allele-specific markers in polyploid species is more complicated than in diploid species. Because, PCR can cause an amplification of multiple, similar sized fragments from more than one genome (Helguera et al., 2000). Therefore, false-positive response can be obtained when the presence of the markers designed for varieties and lines described in the earlier papers (not used in experiments yet) is examined in lines and cultivars with different genetic background. Markers mentioned in the literature must be examined before using them in genetic and breeding programmes.

Therefore, the purpose of the present study was to detect brown (*Lr*) and yellow rust (*Yr*) resistance genes in Azerbaijan wheat germplasm using STS, SSR vs SCAR markers.

MATERIALS AND METHODS

Plants materials. Wheat genotypes differing in resistance to diseases, productivity, architectonics and other physiological traits, collected in the Gene Pool of the Research Institute of Crop Husbandry (Baku) were used as research objects. Plants were cultivated under field conditions.

Extraction of plant DNA. DNA extraction was carried out using the CTAB method with some modifications (Murray & Thompson, 1980).

DNA quantification. After dissolution of the DNA the quantity was determined by optical density (OD) at $\lambda=260$ using the ULTROSPEC 3300 PRO spectrophotometer ("AMERSHAM", USA). Purity of the genomic DNA was determined by the ratio of absorptions at A260/A280. Quality of the DNA was checked on the basis of performance of the extracted DNA samples in 0.8% agarose gel stained with 10 mg/mL of ethidium bromide in 1 × TBE (Tris base, Boric acid, EDTA) buffer. The gel was developed and photographed under ultraviolet light using "Gel Documentation System UVITEK" (UK).

DNA amplification. Polymerase chain reaction was performed by Williams (1990). DNA amplification was performed in a 25 µl reaction mixture volume, containing 10 × buffer, 20 ng of the genomic DNA, 0.2 µM primer, 200 µM of each of the following: dATP, dCTP, dGTP and dTTP, 2.5 mM MgCl₂, and 0.2 units of Taq-polymerase in the incubation buffer. Different primers were used for the test (Table 1).

PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" under the following conditions: 1 cycle - 3 minutes 94 ° C; 38 cycles - 1 min at 94 ° C, an annealing step at variable annealing temperatures depending on the primer

pairs for 1 min, 2 minutes at 72 ° C; the final elongation cycle was performed at 72 ° C for 10 min, then kept at 4 ° C.

PCR products were analyzed by electrophoresis in a 1.2-2% agarose gel in the HR-2025-High Resolution («IBI SCIENTIFIC» U.S.) horizontal electrophoresis machine with addition of ethidium bromide and documented using «Gel Documentation System UVITEK». Dimensions of amplified fragments were determined with respect to 1kb DNA marker. Statistical analysis included binary matrix compilation for each of the primers, in which “presence” (1) or “absence” (0) of fragments with equal molecular weight on the electrophoregram were noted.

RESULTS & DISCUSSION

Detection of brown leaf rust resistance gene *Lr9* in durum and bread wheat genotypes using *STS* marker. STS markers J13/1 and J13/2 were used for *Lr9* gene screening. The objects of the screening for this gene were 78 genotypes (26 genotypes of bread (*Triticum aestivum* L.) and 52 genotypes of durum (*Triticum durum* Desf.) wheat) (Table 2). In PCR profiles of 60% genotypes (16 of them are durum and 31 bread wheat genotypes) 1100 bp fragments were detected, suggesting the presence of the gene *Lr9* on 6B chromosomes of these genotypes. In 40% of genotypes (10 samples of durum and 21 of bread wheat genotypes) the expected fragment was not amplified (Figure 1).

Table 1. Nucleotide sequence of the primers used for the DNA amplification.

Primer description	Gene	Nucleotide sequence	Annealing temperature	Product size, bp
J13/1	<i>Lr9</i>	TCCTTTTATTCCGCACGCCGG	62	1100
J13/2		CCACACTACCCCAAAGAGACG		
SCS123F	<i>Lr19</i>	CCTGATCACCAATGACGATT	60	688
SCS123R		CCTGATCACCTTGCTACAGA	63	737
SCS253F		GCTGGTTCCACAAAGCAAA		
SCS253R		GGCTGGTTCCTTAGATAGGTG		
csLV34a	<i>Lr34</i>	GTGGTTAAGACTGGTGATGG	56	229
csLV34b		TGCTTGCTATTGCTGAATAGT		150
Lr35F	<i>Lr35</i>	AGAGAGAGTAGAAGAGCTGC	55	900
Lr35R		AGAGAGAGAGCATCCACC		
XGWM582F	<i>Yr9</i>	AAGCACTACGAAAATATGAC	60	150
XGWM582R		TCTTAAGGGGTGTTATCATA		
XGM295F	<i>Yr18</i>	GTGAAGCAGACCCACAACAC	60	250
XGM295R		GACGGCTGCGACGTAGAG		

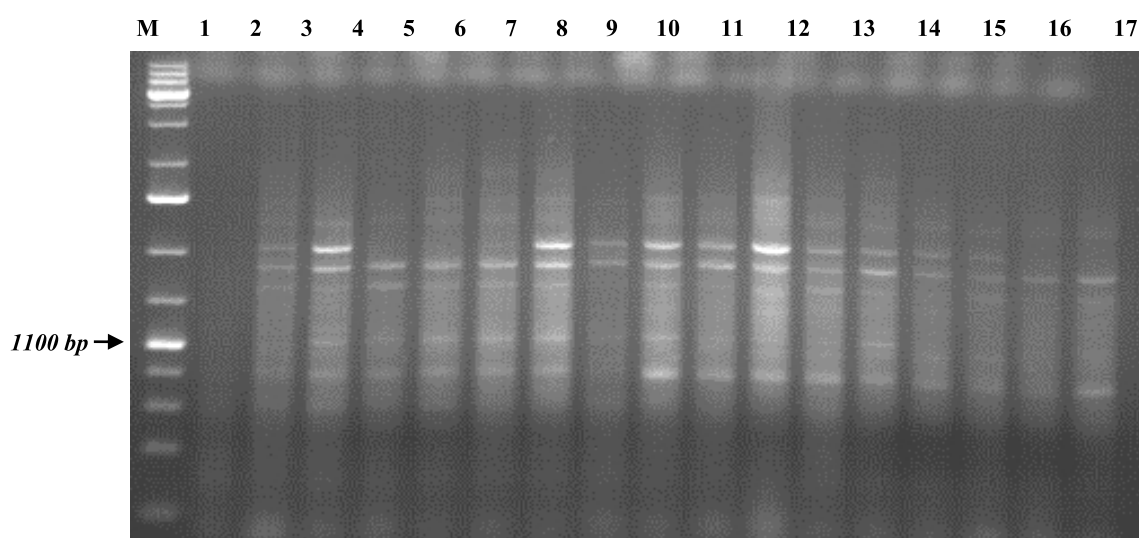


Figure 1. PCR - profiles of wheat plants, for *Lr9*. Arrow indicates the 1100 bp. M - 1kb Plus DNA Ladder.

1 - Vugar, 2 – Alinca 84, 3 - Tartar, 4 - Sharg, 5 – Tartar 2, 6 – Shirvan 5, 7 - Gyzyly bugda, 8- Garabag, 9 - Yagut, 10 - Turan, 11 – Mirbashir 50, 12 – Shirvan 3, 13 - Mugan, 14 - Ag bugda, 15 – Barakatli 95, 16 - Gioaza, 17 – Beltago.

Table 2. Results of the PCR analysis for the gene *Lr9*.

<i>Triticum durum</i> Desf.			
Vugar	-	Mugan	+
Shiraslan 23	-	Ag bugda	+
Alınca 84	+	Kakhraba	-
Tartar	+	Mirvari	-
Sharg	-	Salt tolerant Mapping pop (F7)	+
Gyzyl bugda	+	ID VT 06-DTA (6)	+
Tartar 2	-	S2	+
Garabag	+	Barakatli 95	+
Yagut	+	Kollektivnaya 77	+
Turan	+	Fadda 98	+
Mirbashir 50	+	Gioaza	-
Shirvan 3	+	Beltago	-
Shirvan 5	-	Polonicum	-
<i>Triticum aestivum</i> L.			
Akinchi 84	+	rsi-13 (Shafag 2)	+
Pirshahin 1	-	Pirshahin	+
Gunashli	+	Ugur	+
Dagdas	-	Parzivan 1	-
FARAN Dolc	+	Parzivan 2	+
Renan	+	Shaki 1	-
Avreka	+	S1	-
Pactole	-	Nurlu 99	+
S5	+	Gyrmyzy gul 1	+
TH1	-	Azamatli 95	+
D8 sechme №5	+	Tale 38	+
S4	+	Ruzi 84	+
S3	+	12nd FAWWON №97 (130/21)	-
Sechme SS	-	4th FEFWSN №50 (130/32)	-
Mirbashir 128	+	FO2 N7-A (karlik)	+
Yegana	-	Tigre	+
Zirva 80	+	Bezostaya 1	-
Fatima	+	Kanada 2	+
Azan	+	Sechme sunbul giz	+
Azeri	-	S9	+
Murov	+	Sechme sunbul ag	+
Murov 2	-	Bogdanka	+
Səba	+	Kripsinka	+
Taraggi	+	Polşa	+
Bayaz	+	Podolyanka	-
Shafag	-	Miranovka	-

*Note: [+] – presence of the expected locus, [-] – absence of the locus.

Detection of yellow rust resistance genes *Yr9* and *Yr18* using SSR markers. Screening of the gene *Yr9* was performed using a microsatellite marker XGWM582. Amplifications in 150 bp region are characteristics for this marker (Figure 2). It is interesting that, amplification was successful in 93% of the genotypes, indicating the presence of the gene *Yr* on 1BL chromosome of these genotypes. The exceptions are 4 samples-Azeri, 16th FAWWON-IR (46), 16th FAWWON-IR (90), 16th FAWWON-IR (47). Characteristics fragments for this gene were not synthesized in these genotypes (Table 3).

Molecular marker XGWM 295 was used for the identification of the yellow rust resistance gene *Yr18*. Electrophoretic profiles in figure 5 showed the responsibility of the used marker for the synthesis of fragments in 250 bp region. In 66% of the genotypes, amplification of the expected fragment for the gene *Yr18* was successful (Table 3).

It is known that the gene *Yr18* is genetically inseparable from the leaf rust resistance gene- *Lr34*. These genes are located on the same segment of the chromosome 7D. The locus *Lr34/Yr18* is of great practical interest for solving the discussed problem in bread wheat (Kolmer et al., 2008). Therefore, we performed also screening to test the given locus via the gene *Lr34*.

Assessment of *Lr34* allele status, using the codominant STS *Lr34/Cslv34* marker. Several SSR, STS and CAPS-markers were proposed to identify the gene *Lr34*. However, codominant STS-marker cslV34, closely associated with the locus *Lr34* (0.4 cM), which is a biallelic locus was especially used in MAS programs (Lagudah et al., 2006). Therefore, we used markers *Lr34/cslV34a* and *Lr34/cslV34b* to identify *a* and *b* alleles of the gene *Lr34*. The specific marker for the allele *Lr34/cslV34a* have to lead to the amplification of 229 bp fragments (Figure 4). PCR analysis using this primer revealed corresponding locus only in 21 genotypes (Table 3). This represents approximately 34% of all tested genotypes. №50 (130/32), FO2 N7-A (dwarf) have allele *a* of the brown rust resistance gene - *Lr34*. This allele was not identified in the rest of the genotypes (66%).

According to the PCR profiles obtained with the marker *Lr34/cslV34b*, used for the identification of the *b* allele of *Lr34*, characteristic fragments in 150 bp region were synthesized only in 15% genotypes. In other words, allele *b* of the gene *Lr34* was identified only in 9 (Gunashli, Dagdash, S5, Zirve 80, Gyrmyzy gul 1, Tale 38, Tigre, Bezostaya 1, 29 ES WVT (7)) among 61 genotypes. The electrophoretic analysis of amplified PCR products showed that the existence of allele *b* of the gene *Lr34* was not confirmed in approximately 85% of our wheat genotypes.

Interestingly that in two genotypes – Zirve 80 and Gyrmyzy gul 1 both alleles of the gene *Lr34* were identified. According to the general analysis of the results related to the both markers, 54% of the wheat genotypes do not possess any allele of the gene *Lr34*. The presence of allele state *cslV34b* indicates wheat tolerance to the causative of brown rust, associated with the gene *Lr34*, whereas *cslV34a* indicates the absence of such a tolerance (Karelov et al., 2011).

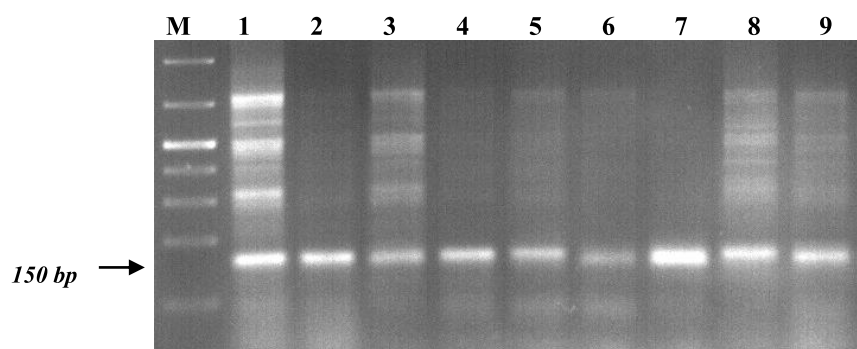


Figure 2. PCR - profiles of *Triticum aestivum* L. plants, for *Yr9*. Arrow indicates the 150 bp. M - molecular weight marker 100 bp. 1 - Pirshahin-1, 2 - Gunashli, 3 - Dagdash, 4 - FARAN Dolc, 5 - Renan, 6 - Avreka, 7 - Pactole, 8 - S5, 9 - TH1.

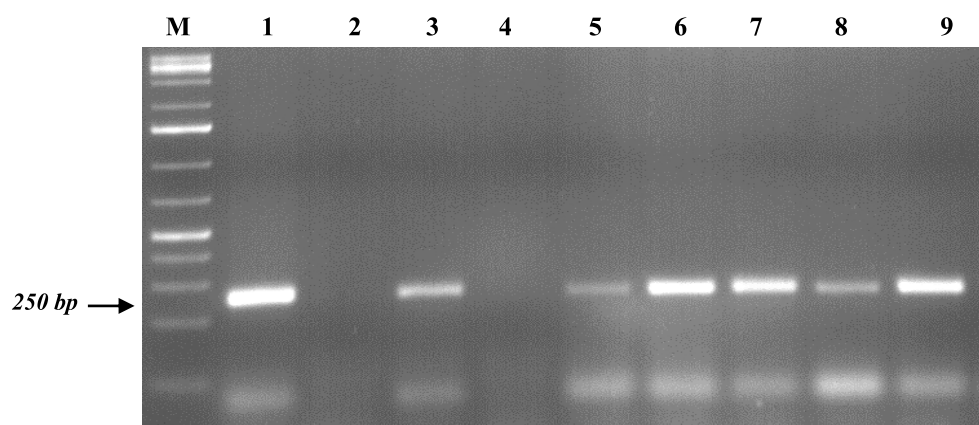


Figure 3. PCR - profiles of *Triticum aestivum* L. plants, for *Yr18*. Arrow indicates the 250 bp. M - molecular weight marker - 100 bp. 1 - Pirshahin-1, 2 - Saba, 3 - Guneshli, 4 - Bayaz, 5 - Dagdash, 6 - FARAN Dole, 7 - Renan, 8 - Avreka, 9 - Pactole.

Table 3. Results of the PCR analysis for the genes *Yr18*, *Yr9*, and *Lr34*.

№	Genotypes	<i>Yr18</i> - 250bp	<i>Yr9</i> - 150bp	<i>Lr34/ csLV34a</i> - 229bp	<i>Lr34/ csLV34b</i> - 150bp
1	2	3	4	5	6
1.	Pirshahin 1	+	+	+	-
2.	Gunashli	+	+	-	+
3.	Dagdash	+	+	-	+
4.	FARAN Dolc	+	+	+	-
5.	Renan	+	+	+	-
6.	Avreka	+	+	+	-
7.	Pactole	+	+	+	-
8.	S5	+	+	-	+
9.	TH1	+	+	+	-
10.	D8 sechme №5	+	+	+	-
11.	S4	+	+	+	-
12.	S3	+	+	+	-
13.	Sechme SS	+	+	-	-
14.	Mirbashir 128	+	+	-	-
15.	Yegane	+	+	-	-
16.	Zirve 80	+	+	+	+
17.	Fatima	+	+	-	-
18.	Azan	+	+	+	-
19.	Azeri	+	+	-	-
20.	Murov	+	+	-	-
21.	Murov 2	+	+	+	-
22.	Saba	-	+	+	-
23.	Taraggi	+	+	+	-
24.	Beyaz	-	+	-	-

Continued Table 3

1	2	3	4	5	6
25.	Shafag	+	+	+	-
26.	rsi-13 (Shafaq-2)	+	+	-	-
27.	Pirshahin	+	+	-	-
28.	Ugur	+	+	-	-
29.	Perzivan 1	+	+	-	-
30.	Perzivan 2	-	+	-	-
31.	Shaki 1	-	+	+	-
32.	S1	-	+	-	-
33.	Nurlu 99	-	+	-	-
34.	Gyrmyzy gul 1	-	+	+	+
35.	Azamatli 95	-	+	+	-
36.	Tale 38	+	+	-	+
37.	Ruzi 84	+	+	-	-
38.	12nd FAWWON №97 (130/21)	+	+	+	-
39.	4th FEFWSN №50 (130/32)	+	+	+	-
40.	FO2 N7-A (karlik)	+	+	+	-
41.	Tigre	+	+	-	+
42.	Bezostaya 1	+	+	-	+
43.	Kanada 2	-	+	-	-
44.	Sechme sunbul giz	-	+	-	-
45.	S9	+	+	-	-
46.	Sechme sunbul ag	-	+	-	-
47.	Bogdanka	+	+	-	-
48.	Kripsinka	+	+	-	-
49.	16th FAWWON-IR (61)	-	+	-	-
50.	16th FAWWON-IR (46)	+	-	-	-
51.	16th FAWWON-IR (52)	-	+	-	-
52.	16th FAWWON-IR (90)	+	-	-	-
53.	16th FAWWON-IR (47)	+	-	-	-
54.	29 ES WVT (7)	-	+	-	+
55.	29 ES WVT (26)	-	+	-	-
56.	29 ES WVT (38)	-	+	-	-
57.	29 ES WVT (30)	-	+	-	-
58.	16 SAWWVT (29)	-	+	-	-
59.	16 SAWWVT (34)	-	+	-	-
60.	39 IBWSN (97 №)	-	+	-	-
61.	11st IWWT-R (9816 №)	-	+	-	-

*Note: [+] – presence of the expected locus, [-] – absence of the locus.

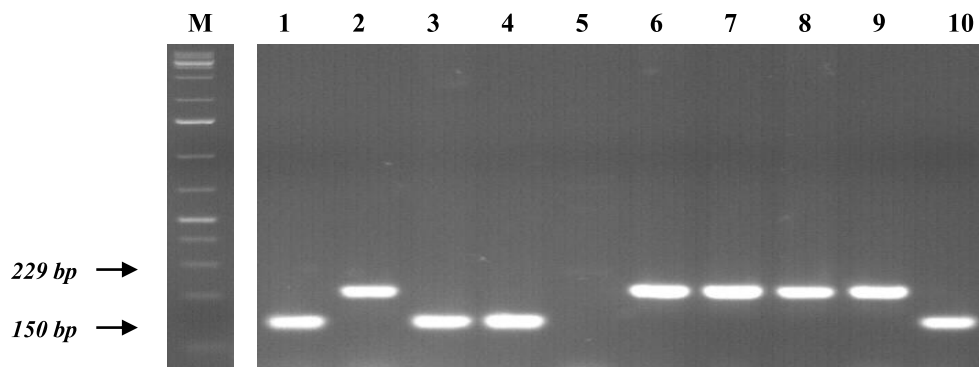


Figure 4. PCR - profiles of *Triticum aestivum* L. plants for *Lr 34*. Arrow indicates the 150 bp and 229 bp. molecular weight marker - 100 bp. 1 - Dagdash, 2 – Pirshahin 1, 3 - Guneshli, 4 - S5, 5 - Saba, 6 - FARAN Dole, 7 - Renan, 8 - Avreka, 9 - Pactole, 10 – Tale 38.

We have compared the results obtained for the brown rust resistance gene *Lr34* as well as for the yellow rust resistance gene *Yr18*. As the gene imparting resistance has *b* allele, comparison was performed according to this allele. Only 7 genotypes from the tested 61 showed a positive result for the both genes. Therefore, we can confidently say that

the locus *Lr34/Yr18* is present on 7D chromosomes of the genotypes Gunashli, Tigre, Tale 38, S5, Dagdash, Zirve 80, Bezostaya-1. Negative results were obtained for 19 genotypes (approximately, 33%) for the both genes. In other words, the locus *Lr34/Yr18* related to resistance to both brown and yellow rust is absent in these genotypes. Positive

results for *b* allele of the gene *Lr34* and negative results for the gene *Yr18* were obtained only for two genotypes. The amplification of the characteristic fragments for the gene *Yr18* was successful in 54% of the genotypes and specific fragments for *b* allele of the gene *Lr34* were not synthesized. It should be noted that, some of these genotypes, lacking this locus are resistant to deleterious diseases in field conditions. Apparently, the resistance of such genotypes is determined by other genes.

Genetic diversity of modern bread wheat varieties (*Triticum aestivum* L.) based on the genes of resistance to yellow rust (*P. striiformis* Westend. f. sp. *tritici*) and brown rust (*Puccinia tritici* Erikss.) is small. At best 1-2 and sometimes 3 genes are identified in these varieties (Gaynullin, 2008, Sibikeev, 2002, Mesterhazy et al., 2000). The protection strategy of bread wheat from rust, which is the most common and harmful disease, includes several directions having their pros and cons. From a genetic point of view, the search for the new resistance genes *Lr* and *Yr*, as well as creation of varieties that combine race-specific and nonspecific resistance, provide effective and long-term resistance to these infections (Sehgal et al., 2012). Therefore, finding sources and donors of bread wheat to this disease, as well as identifying combinatory of the genes *Lr* and *Yr*, providing resistance to brown and yellow rust, remains an urgent problem in the selection of bread wheat.

Determination of brown leaf rust resistance genes *Lr19* and *Lr35* using SCAR markers. *Lr19* localized on the 7D chromosome is one of the few widely effective genes conferring resistance against brown leaf rust in wheat. (Gupta et. al., 2006) Foreign *Lr19* gene demonstrated efficacy against all pathotypes of leaf rust in South Africa (Prins et al., 1997) India (Tomar and Menon, 1998), Europe (Mesterhazy et al., 2000) and Canada (McCallum and Seto-Goh, 2003). The *Lr19* translocation is associated with deleterious agronomic effects and as a result modified forms of the translocation have been derived by different researchers in an attempt to remove the genes responsible. It was reported that *Lr19* was associated with increases in grain yield. Aerial biomass was also increased when *Lr19* was introgressed, although differences were not associated with improved light interception (indirectly measured) or radiation use efficiency (RUE). The physiological basis of the increased biomass and the mechanisms causing increased number of grains per spike, in terms of dynamic of floret development, are not completely understood.

Lr-19 translocation originally produce by Sharma and Knott (1966) when they transform leaf rust resistance genes 7e 11 chromosome of *Thinopyrum ponticum* a long arm of chromosome 7 D of

common wheat. Heurta-Espino and Singh (1994) reported first virulence in *Puccinia Triticinan* to *Lr-19* and it is an effective source of leaf rust resistance worldwide. The cut-of point of *Lr-19* translocation is located in the middle of long arm of chromosome 7D and find that the distal half of 7 D was replaced by *Thinopyrum Chromatinv*. During meiosis *Thinopyrum* segment 7DL does not pair with homologous wheat segment, complicating attempts to study linkage relationship or to recombine its genes (Sehgal, 2012).

Despite the virulence for the *Lr19* gene, there are reports that in the last decade it has demonstrated high efficacy in wheat cultivation areas (Huerta-Espino and Singh, 1994; Sibikeev et al., 1997). High efficacy of the *Lr19* gene in Asia, Australia and Europe indicates that this gene can be used in combination with other *Lr* genes for long-term resistance to leaf rust all over the world (Roelfs, 1988; Pink, 2002)

On this basis, the objective of this study was to determine the presence of the *Lr19* gene in different wheat genotypes using SCAR markers. DNA samples were screened using two SCAR molecular markers bound to a known *Lr19* gene of resistance to brown leaf rust. SCAR markers are polymorphic and amplified unique bands linked to the *Lr19* gene (Gupta et al., 2006). This gives a possibility of using these markers in marker-assisted breeding for *Lr19* gene.

Figure 5 reflects the PCR profiles performed using SCS123 F/R molecular marker. This marker must lead to the amplification of fragments of 688 bp in size. As a result of PCR test with this primer the locus of the 688 bp region was detected only in 48 genotypes. This is approximately 79% of all investigated genotypes.

Fragment linked to the SCS123F/R marker was not synthesized in the following genotypes - Pirshahin-1, Pactole, 8th WVEERYT (32 №), 3 RBWYT (521 №), 3 RBWYT (536 №), 11st IWWYT-R (9816 №), S5, 16th FAWWON-IR (90), 16th FAWWON-IR (47), S1, Nurlu-99 Kyrmyzygul-1, 12nd FAWWON № 97 (130/21).

The second SCAR marker linked to the studied *Lr19* gene of resistance to brown leaf rust was SCS253 F/R. Amplification products with the use of this marker are detected in the 737 bp region. As can be seen from Figure 6, the expected fragment in the 737 bp region was synthesized in only in 53 of 61 genotypes, in other words, in approximately 87% of all investigated genotypes. Fragments specific for the SCS 253F/R SCAR marker were not amplified in the following genotypes - 3 RBWYT (521 №), Zirve 80, Gyrmyzy gul 1, S1, Azamatli 95, Tale 38, Ruzi 84 and 12nd FAWWON № 97 (130/21).

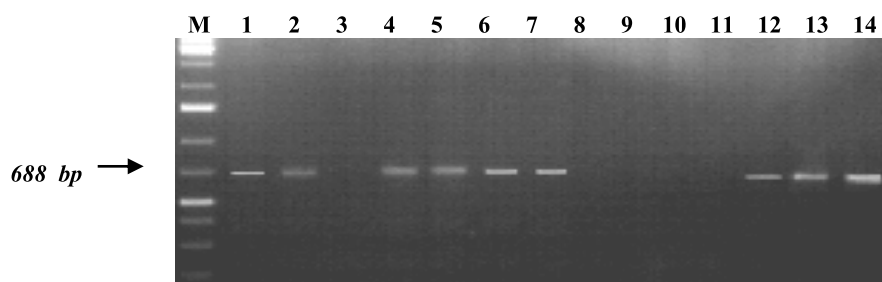


Figure 5. PCR-profiles of *Triticum aestivum* L. plants induced by SCS123F/R primer.

Arrow indicates the 688 bp. M- 1 kb DNA ladder. 1 – 10 SAWVT (11 №), 2 – 3th FAWWON (117 №), 3 – 8th WVEERYT (32 №), 4 – 4th RWVT-LRCA (89 №), 5 – 14th FAWWON (86 №), 6 – 8th WONSA (65 №), 7 – 9th WON-SA (27 №), 8 – 3 RBWYT (521 №), 9 – 12nd FAWWON №97 (130/21), 10 – 3 RBWYT (536 №), 11 – 3 RBWYT (518 №), 12 – 39 IBWSN (113 №), 13 – 14 SAWYT (49 №).

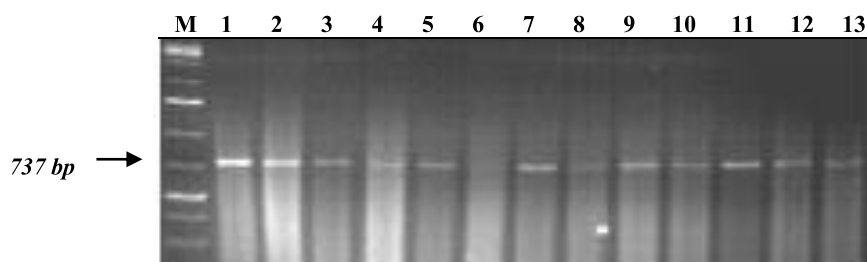


Figure 6. PCR-profiles of *Triticum aestivum* L. plants induced by the SCS253F/R primer.

Arrow indicates the 737 bp. M – 1kb DNA ladder. 1 - 39 IBWSN (97 №), 2 - 11st IWWYT-R (9816 №), 3 – S5, 4 – Mirbashir 128, 5– Yegane, 6 – Zirve 80, 7 – Fatima, 8 – Aran, 9 – Azeri, 10 – Murov, 11 – Murov-2, 12 – Saba, 13 – Taraggi.

Comparative analysis of PCR profiles obtained with the use of both SCAR markers demonstrates, that the results are the same in 82% of genotypes: specific amplification fragments were identified in 45 genotypes with the use of both SCS123F/R and SCS253F/R markers, which indicates that the *Lr19* gene of resistance to brown leaf rust is present on 7D chromosomes of these genotypes. The existence of the *Lr19* gene has not been proven in 5 of 61 genotypes used, because specific fragments amplified with any of the applied markers were not identified in these genotypes.

The results obtained with different markers did not match in 18% of genotypes. After using the SCS123F/R marker, nine genotypes (Pirshahin-1, Pactole, 8th WVEERYT (32 №), 3 RBWYT (536 №), 11st IWWYT-R (9816 №), S5, 16th FAWWON-IR (90), 16th FAWWON-IR (47), Nurlu 99) did not match, i.e. fragments in the 688 bp region specific for the SCS123F/R marker were not synthesized in these genotypes, on the contrary, the 737 bp fragments, linked with the SCS253F/R marker, were amplified. And this kind of mismatch was detected in three genotypes (Zirve 80, Azamatli 95, Ruzi 84) with the use of the SCS253F/R marker, in other words, amplification products specific for the SCS253F/R marker were absent in

these genotypes, on the contrary, synthesis of PCR profiles specific for the SCS123F/R marker has successfully been performed.

The absence of marker components with the *Lr19* gene in these samples may be due to an incomplete linkage of the marker and the gene (Tirishkin, 2006).

Attention is drawn to the fact that resistance and high sensitivity to brown leaf rust are observed among the genotypes in which amplification products have not been revealed, thus indicating the absence of the *Lr19* gene.

Gyrmyzy gul-1 wheat genotype in field conditions also demonstrates high susceptibility to the brown rust pathogen and is completely affected by the *Puccinia recondite* f. sp. *tritici* fungus. The genotypes called 3 RBWYT (521 №), S1 and Tale-38 in field conditions are estimated as moderately resistant to this disease. It is interesting that the 12nd FAWWON № 97 (130/21) genotype actually demonstrates high resistance to this harmful disease, despite the absence of the *Lr19* gene. Apparently, the resistance of this genotype may be caused by other *Lr*-genes.

The marker *Lr35F/Lr35R* was used to identify the gene *Lr35*. The analysis was performed on 61 bread (*Triticum aestivum* L.) wheat genotypes.

When using the marker *Lr35F/Lr35R*, specific fragments had to be synthesized at 900 bp region (the figure is not presented). Fragments were not visualized at this region in the obtained electrophoretic profiles. In other words, using this marker, the existence of the gene *Lr35* on 2B chromosomes have not been proven.

CONCLUSION

The study of the genetic basis of plant resistance, the search for effective genes and their introduction into the culture of wheat significantly prevent the spread of the epiphytotic disease and stabilize the grain yield capacity. Development and deployment of cultivars with host genetic resistance is the most ecofriendly way to reduce the losses. The use of modern molecular-genetic techniques greatly accelerates the process of the identification of genotypes resistant to diseases and the creation of disease-resistant varieties. Molecular markers are widely used for the investigation of the bread wheat genome structure, identifying and mapping genes responsible for expression of the useful properties, as well as for the isolation and cloning of genes for studying their controlled properties and transmission them to other varieties (i.e. for genetic transformation). Thus, the use of molecular markers in breeding allows us to obtain information on the sign at the early stages of development, without waiting for the phenotypic expression of feature, simplifies testing resistance to various diseases, requiring thorough assessment by traditional research methods.

The wheat cultivars are of different types and become susceptible to different types of rust because it has narrow genetic bases for resistance. The evolution rates of pathogens are very fast and rapid. So, it is necessary to find out new and better sources for resistance. The genetic resistance is important to control many phytopathogenic epidemics. The wheat production has been dependent on the use and development of rust resistance genotypes having well characterized and diverse genes. It is also concluded that in wheat certain and different combinations of genes give long lasting and better resistance for rust diseases than given by any individual genes.

The obtained results can be used in breeding and genetic programs on creation of forms resistant to leaf rust pathogen populations in Azerbaijan. Thus, information about the existence of effective *Lr* and *Yr* genes in adapted varieties that can be used as donors for resistance, and usage of these distinct genes or by pyramiding of different resistance genes in the genotype can significantly im-

prove the efficiency of breeding of resistant varieties, thus assisting to avoid the creation of varieties that are genetically homogeneous. This information will serve as a foundation for plant breeders and geneticists to develop durable rust-resistant wheat varieties through marker-assisted breeding or gene pyramiding.

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Azərbaycan Buğda Germplazmasında Qonur Pasa Qarşı *Lr9*, *Lr19*, *Lr34*, *Lr35* və Sarı Pasa Qarşı *Yr9*, *Yr18* Davamlılıq Genlərinin Molekulyar Analizi

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Pas dənin məhsuldarlığını aşağı salan təhlükəli göbələk xəstəliyidir. Pas xəstəliklərinə nəzarət üçün pasa davamlı sortların becərilməsi səmərəli və ekoloji baxımdan daha təhlükəsiz yanaşmadır. Müxtəlif molekulyar metodlardan istifadə etməklə, yumşaq və bərk buğda genotiplərində və onların yabanı növlərində hələlik qonur pasa qarşı 81 və sarı pasa qarşı 53 davamlılıq geni aşkar edilmişdir. Tədqiqat işinin əsas məqsədi Əkinçilik ET İnstitutunun genofondunda toplanmış buğda nümunələrində *Lr* və *Yr* genlərinin mövcudluğunu müəyyənləşdirmək olmuşdur. *Lr9*, *Lr19*, *Lr34*, *Lr35* və *Yr9* və *Yr18* genləri müxtəlif molekulyar markerlərlərdən istifadə etməklə analiz olunmuşdur. PZR profillərdə 1100 bp uzunluğunda spesifik fraqmentlərin olması ilə tədqiq edilən 78 genotipdən 60%-nin 6B xromosomlarında *Lr9* geninin mövcudluğu müəyyən edilmişdir. SCS123 və SCS253 SCAR markerlərin köməyiylə *Lr19* geninin identifikasiyası zamanı 45 genotipdə müsbət nəticə müşahidə edilmişdir. *Lr43* geninin allel vəziyyəti *Lr34/csLV34a* və *Lr34/csLV34b* markerlərin köməyiylə tədqiq edilmişdir. Molekulyar analiz 21 genotipdə *Lr34* geninin *a* allelini, 9 genotipdə isə *b* allelini üzə çıxarmışdır. İki genotipdə isə *Lr34* geninin hər iki alleli müşahidə edilmişdir. Genotiplərin 54%-də *Lr43* geninin heç bir alleli müəyyən edilməmişdir. 61 genotipin PZR profillərində *Lr35* geni üçün xarakterik fraqmentlər aşkar edilməmişdir. 40 genotipin elektroforetik profillərində *Yr18* geni üçün diaqnostik 250 bp fraqmentlər müşahidə edilmişdir. 4 genotip istisna olmaqla, digər genotiplərin hamısında *Yr9* geni üçün xarakterik 150 bp sahədə fraqmentlər amplifikasiya olunmuşdur. Bu nəticələr Azərbaycanda buğdanın pas xəstəliklərinə nəzarət edilməsi və pasa davamlı buğda sortlarının yaradılmasında əsas kimi istifadə oluna bilər.

Açar sözlər: Buğda, qonur pas, sarı pas, *Lr9*, *Lr19*, *Lr34*, *Lr35*, *Yr9*, *Yr18*, PCR, STS, SSR, SCAR

Молекулярный Анализ Генов Устойчивости *Lr9*, *Lr19*, *Lr34*, *Lr35* к Бурой Ржавчине И *Yr9*, *Yr18* к Желтой Ржавчине в Гермплазме Пшеницы Азербайджана

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Ржавчина – это опасное грибковое заболевание, понижающее урожайность зерна. С целью усиления контроля над заболеванием ржавчиной наиболее выгодным и безопасным с экологической точки зрения подходом является выращивание устойчивых к этому заболеванию сортов. С помощью различных молекулярных методов на сегодняшний день в генотипах мягкой и твердой пшеницы и их диких видов выявлены 81 ген, устойчивый к бурой ржавчине, и 53 гена, ответственных за устойчивость к желтой ржавчине. Основная цель работы заключалась в установлении наличия генов *Lr* и *Yr* в образцах пшеницы, взятых из генофонда НИ Института земледелия. С использованием различных молекулярных маркеров были изучены четыре *Lr* гена – *Lr9*, *Lr19*, *Lr34*, *Lr35* и два *Yr* гена – *Yr9* и *Yr18*. Благодаря обнаружению в ПЦР профилях специфических фрагментов длиной 1100bp, у 60%-ов из исследованных 78 генотипов было выявлено наличие в 6B хромосоме гена *Lr9*. При идентификации гена *Lr19* с помощью SCAR маркеров SCS123 и SCS253 был получен положительный результат в 45 генотипах. Аллельное состояние гена *Lr43* было исследовано с помощью маркеров *Lr34/csLV34a* и *Lr34/csLV34b*. Молекулярным анализом в 21 генотипе была выявлена *a* аллель, а в 9 генотипах – *b* аллель гена *Lr34*. В двух же генотипах наблюдались обе аллели гена *Lr34*. У 54%-ов генотипов аллели гена *Lr43* не выявлялись. В ПЦР профилях 61 генотипа не обнаруживались характерные для гена *Lr35* фрагменты. На электрофоретических профилях 40 генотипов наблюдались диагностические для гена *Yr18* фрагменты длиной 250 bp. Во всех генотипах, за исключением четырех, амплифицировались характерные для гена *Yr9* фрагменты длиной 150 bp. Наши результаты могут служить основанием для выведения продолжительно устойчивых к ржавчине сортов пшеницы и усиления, тем самым, контроля над заболеванием ржавчиной пшеницы в Азербайджане.

Ключевые слова: Пшеница, бурая ржавчина, желтая ржавчина, *Lr9*, *Lr19*, *Lr34*, *Lr35*, *Yr9*, *Yr18*, ПЦР, STS, SSR, SCAR

Diurnal Changes of the Titratable Acidity in A New CAM Plant, *Sedum caucasicum* Leaves

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Determination of the photosynthetic pathways of the plants in the studied areas would be very useful for evaluation of the modern vegetation and prediction of changes. Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that features nocturnal CO₂ uptake, facilitates increased water-use efficiency. A large variation in CAM has been found within the genus *Sedum*. C₃ species, CAM constitutive species and CAM inducible species under water stress or salinity have been recognized. Diurnal acidity cycle in *Sedum caucasicum* species which is considered endemic in Caucasus occurs similar to that of in CAM plants regardless if they are grown in a greenhouse or naturally. The obtained results confirm that *Sedum caucasicum* is an obligate CAM plant.

Keywords: CAM photosynthesis, *Sedum caucasicum*, titratable acidity, photochemical efficiency

INTRODUCTION

Crassulacean acid metabolism (CAM) is a water-conserving mode of photosynthesis and one of three photosynthetic pathways which occurs in 7% of vascular plant species (Winter and Smith, 1996), many of which dominate the plant biomass of arid, marginal regions of the world. CAM is a modification of the basic C₃ pathway and represents CO₂-concentrating mechanisms that elevate CO₂ around Rubisco and suppress photorespiration. This is achieved in two principal phases separated in time. At night, atmospheric CO₂ is incorporated by phosphoenolpyruvate carboxylase (PEPC) via oxaloacetate into malic acid, which accumulates in the large vacuoles of chloroplast-containing mesophyll cells. During the following light period, malic acid is released from the vacuoles and decarboxylated, and the CO₂ thus liberated is refixed by Rubisco and reduced in the Calvin cycle (Osmond, 1978; Winter & Smith, 1996). Decarboxylation of malate generates high intercellular CO₂ and is associated with stomatal closure, minimizing water loss in the middle of the day when evaporative demand is highest. The net result of CAM is an improvement in water use efficiency generally 6-fold higher than for C₃ plants and 3-fold higher than for C₄ plants under comparable conditions (Nobel 1996).

CAM may operate in different modes: (1) obligate CAM, with high nocturnal acid accumulation (ΔH^+) and CO₂ fixation; (2) facultative or inducible CAM, also known as C₃-CAM, with a C₃ form of CO₂ fixation and nil (ΔH^+) in the non-induced state, and small nocturnal CO₂ fixation and ΔH^+ in the induced state; (c) CAM-cycling, with daytime CO₂

fixation and ΔH^+ but no nocturnal stomatal aperture; and (d) idling, with small ΔH^+ and stomatal closure during the entire day and night in severely stressed plants (Ana Herrera, 2009). On the basis of the magnitude of ΔH^+ obligate CAM, can be strong and weak CAM form (Winter and Holtum, 2002; Silvera et al., 2005; Holtum et al., 2007). In facultative species, CAM may be induced by factors such as drought (Borland and Griffiths, 1990; Herrera et al., 1991; Olivares et al., 1984), salinity (Winter and von Willert, 1972), photoperiod (Brulfert et al., 1988), high photosynthetic photon flux (PPF) (Maxwell, 2002), nitrogen deficiency (Ota, 1988) and phosphorus deficiency (Paul and Cockburn, 1990), among others.

Sedum is a large genus of flowering plants in the family *Crassulaceae*, members of which are commonly known as stonecrops. The genus has been described as containing up to 600 species updated to 470. There are 22 species of the genus *Sedum* in the flora of Azerbaijan. *Sedum caucasicum* (Grossh.) *Hylotelephium caucasicum*, grows on dry stony and limestone slopes in mixed deciduous forests of the Caucasus (Figure 1). The plant is herbaceous, perennial, with the aerial part completely dying for the winter. Roots are thickened, fusiform. Stems are simple, among several, raised, strong, straight, green or dark purple. Due to the arrangement of leaves plants can use solar energy with maximum efficiency.

Work presented in this paper aimed at investigating the presence of CAM in *S. caucasicum*. Investigations involved field and greenhouse studies of diurnal changes in titratable acidity.



Figure 1. *S. caucasicum* plants at anthesis under natural conditions.

MATERIALS AND METHODS

The study site was located at the Lesser Caucasus Mountain (Tovuz region) in the north-west of Azerbaijan (40°59'31.92"N, 45°37'44.04"E) and at the Main Caucasus Mountain (Ismayilli region) in the north-eastern part of Azerbaijan (40°51'11.16"N, 48°23'35.16"E).

Plant material. Mature *Sedum caucasicum* plants were used as plant material.

Titrateable acidity was determined in leaves of *Sedum caucasicum* at different times of. Extracts were prepared by grinding 0.5 gr frozen tissue in distilled water and titrating them to a pH 7.0 end point with 0.01 N NaOH (FJ.Castillo,1996)

Determination of RWC. To obtain the leaf RWC, fresh mass, mass after rehydration (equilibrated at 100% RH, at 4°C in distilled water for 24 h in the dark) and dry mass were determined (Castillo,1996).

RWC was calculated as follows:

$$RWC = (FW - DW) / (SW - DW) \times 100$$

where FW is the fresh weight, DW is the dry weight, and SW is the mass after rehydration.

Fluorescence induction was measured with intact leaves, at ambient temperature with a portable fluorimeter (Mini-PAM fluorometer, WALZ, Germany). Fluorescence parameters were as follows: F_0 , initial fluorescence; F_m , maximum fluorescence, F_v , variable fluorescence ($F_m - F_0$; Bolhar-Norden-kampf et al., 1989). Measured chlorophyll fluorescence parameters included F_v/F_m , and Φ PSII, reflecting the efficiency of PSII antenna and the quantum yield of PSII, respectively.

RESULTS AND DISCUSSION

CAM in *Sedum caucasicum* L. (*Crassulaceae*) was analysed by studying diurnal acidity changes depending on environmental factors both in natural habitat of the plant and in phytotron. The extent of

CAM at these 4 various stages of drought was assessed by measuring the overnight accumulation of titrateable acidity. Diurnal acidity changes were observed under natural as well as controlled condition. The quantity of titrateable acid which was very high in the early morning, decreased during the day and reached a minimum value at 17⁰⁰-18⁰⁰. At the early hours of night (at 22⁰⁰) average acidity was 1/3 of the acidity observed in the morning hours. Total titrateable acidity in flowers and green stems under natural conditions was less than in leaves and it did not change pronouncedly during the day. Despite the fact that, the quantity of titrateable acidity was less in juvenile leaves compared with young and middle-aged leaves of the plants studied under both natural conditions, cyclic changes of acidity occurred in juvenile leaves contrary to flowers and green stems of young and middle-aged ones (Table 1).

Acidity of old leaves, completed their vegetation, was relatively less than that of young and middle-aged leaves. It suggests that the rate of photosynthesis and the intensity of metabolism are positively correlated with the titrateable acidity. The quantity of titrateable acidity in *S. caucasicum* is much less in initial leaves, flowers (mature and immature) and stems compared with leaves and this amount remains unchanged during the day. This shows that CAM photosynthesis does not occur in flowers, stem and initial leaves of *S. caucasicum*. However, quantity of acidity and its diurnal changes have similar patterns in young and middle-aged leaves. This confirms that there is no significant difference in the rate of CAM photosynthesis between these leaves.

The titrateable acidity in different aged leaves of the *S. caucasicum* plant cultivated in controlled conditions was lower in older leaves than in young and middle aged plants (Table 2). Thus, in young and middle aged leaves the quantity of titrateable were $\Delta H^+ = 120.0 \pm 0.2 \mu\text{mol g}^{-1}$ and $\Delta H^+ = 124.0 \pm 0.2 \mu\text{mol g}^{-1}$ respectively, and $\Delta H^+ = 106.0 \pm 0.15 \mu\text{mol g}^{-1}$ in older leaves.

Table 1. Diurnal changes in the titratable acidity in above-ground organs and leaf tiers of *S. caucasicum* under natural conditions.

Plant organs	8 ⁰⁰	12 ⁰⁰	16 ⁰⁰	20 ⁰⁰	24 ⁰⁰
Initial leaf	44.0±0.2	42.0±0.1	38.0±0.2	40.0±0.1	44.0±0.1
1 leaf	114.0±0.1	46.0±0.02	26.0±0.01	20.0±0.2	60.0±0.2
2 leaves	122.0±0.2	76.0±0.02	26.0±0.01	22.0±0.2	70.0±0.3
3 leaves	126.0±0.25	88.0±0.03	24.0±0.01	20.0±0.1	70.0±0.3
4 leaves	130.0±0.3	96.0±0.03	24.0±0.01	20.0±0.2	65.0±0.3
5 leaves	124.0±0.3	88.0±0.03	24.0±0.01	20.0±0.2	60.0±0.3
6 leaves	124.0±0.3	60.0±0.02	22.0±0.01	18.0±0.3	60.0±0.3
Flower	40.0±0.01	38.0±0.01	38.0±0.01	40.0±0.1	40.0±0.1
Stem	36.0±0.01	36.0±0.01	36.0±0.01	40.0±0.1	40.0±0.1

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight

Table 2. Changes in the titratable acidity in various aged leaves of *S. caucasicum*

	8:00	12:00	16:00	20:00	24:00	04:00
Young aged leaves	134.0±0.3	50.0±0.1	14.0±0.05	14.0±0.05	60.0±0.1	110.0±0.2
Middle aged leaves	140.0±0.4	50.0±0.1	16.0±0.05	16.0±0.05	60.0±0.1	110.0±0.2
Juvenile leaves	126.0±0.3	40.0±0.1	20.0±0.05	20.0±0.05	50.0±0.1	100.0±0.2

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight.

Table 3. Changes in the titratable acidity in various parts of *S. caucasicum* leaves.

Part of leaf	8 ⁰⁰	12 ⁰⁰	16 ⁰⁰	20 ⁰⁰	24 ⁰⁰	04 ⁰⁰
Top part of the leaf	134.0±2.5	55.0±1.4	14.0±0.9	14.0±0.9	60.0±1.4	110.0±2.2
Middle part of the leaf	134.0±2.4	65.0±1.4	16.0±0.9	16.0±0.9	50.0±1.5	110.0±2.2
Bottom part of the leaf	130.0±2.6	50.0±1.4	20.0±0.9	20.0±0.9	50.0±1.5	110.0±2.2

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight.

Table 4. Changes in the titratable acidity in different conditions in *S. caucasicum* leaves.

Plant	07 ⁰⁰	11 ⁰⁰	15 ⁰⁰	19 ⁰⁰	23 ⁰⁰	03 ⁰⁰	07 ⁰⁰
20-24°C (Natural conditions)	124.0±2.6	90.0±1.6	20.0±1.2	18.0±1.2	60.0±0.1	116.0±2.2	120.0±2.2
30-32°C (Absheron peninsula)	112.0±1.8	50.0±1.6	16.0±1.2	16.0±1.6	65.0±0.1	128.0±2.6	110.0±1.8

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight.

The changes in the quantity of titratable acidity were studied in various parts of *S. caucasicum* leaves (Table 3). The total acidity in the different parts of the leaves were approximately the same and dawn was $\Delta\text{H}^+=120.0\pm0.2 \mu\text{mol g}^{-1}$ and their changes have similar patterns.

The quantity of titratable acidity changed differently in plants grown under natural as well as controlled conditions depending on the environmental parameters. Decline in acidity started at 11:00-12:00 in plants grown under natural conditions (20-24°C). As the temperature of the Absheron peninsula is higher than 30°C in June-July, the decline in the titratable acidity of these plants begins earlier (Table 4).

Dependence of the quantity of titratable acidity on temperature, humidity of the environment and time in the *S. caucasicum* plant under outdoor conditions in June is presented in Table 5.

As seen in the table high environmental temperature has caused a sharp decline in the quantity

of titratable acidity since the early morning hours. Changes in the quantity of the titratable acidity and RWC were also measured in the *S. caucasicum* plants under normal and drought conditions (Figure 2).

Table 5. Changes in the titratable acidity dependence on temperature, humidity of the environment in *S. caucasicum* leaves.

Time, hour	T, °C	RH	Titratable acidity, $\mu\text{mol H}^+\text{g}^{-1}$ fresh weight
07 ⁰⁰	30	45	150.0±2.7
09 ⁰⁰	31	54	128.0±2.6
11 ⁰⁰	33	41	100.0±2.4
13 ⁰⁰	36	44	36.0±0.9
15 ⁰⁰	32	48	24.0±0.8
17 ⁰⁰	30	56	20.0±0.8
19 ⁰⁰	27	63	36.0±0.8
21 ⁰⁰	27	65	56.0±0.9
23 ⁰⁰	26	70	64.0±0.9
01 ⁰⁰	25	73	84.0±0.9
03 ⁰⁰	24	72	98.0±0.9
05 ⁰⁰	24	61	170.0±2.9

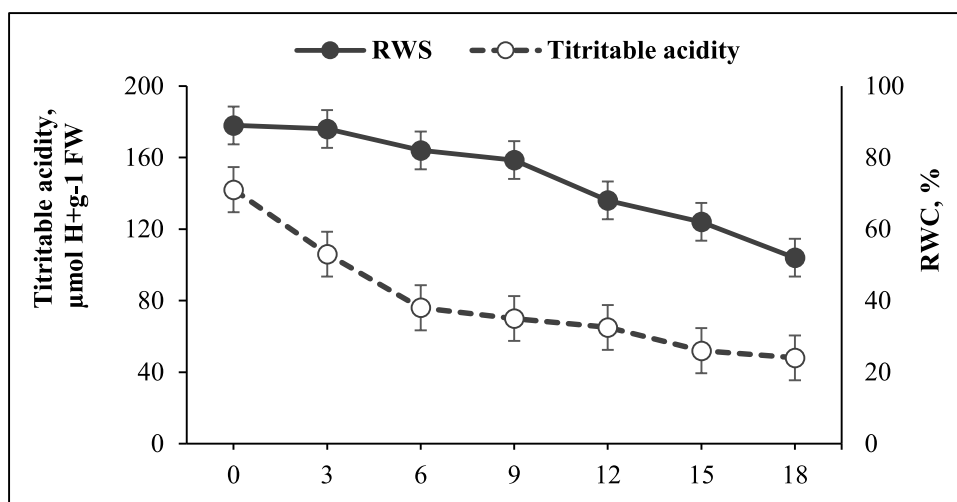


Figure 2. Changes in RWC and titratable acidity depending on the duration of the drought stress, in mature leaves of the *S. caucasicum* plants during early morning hours.

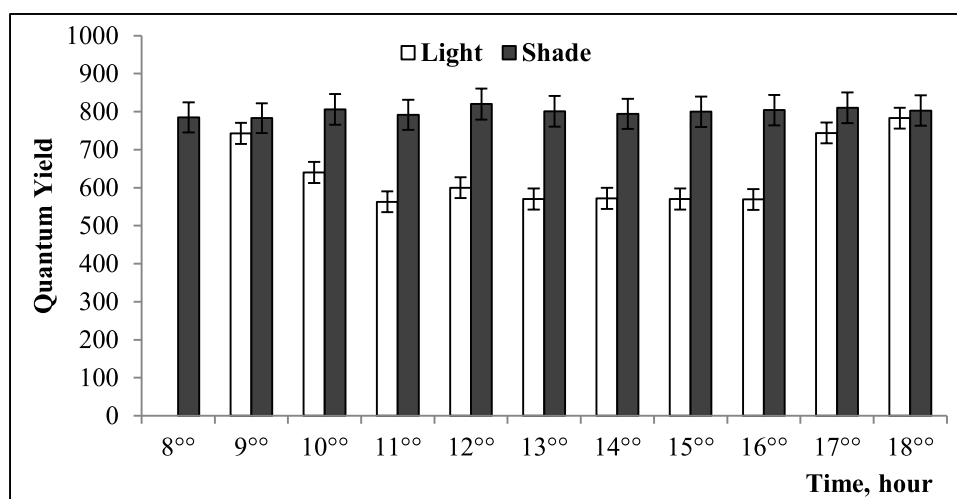


Figure 3. Changes in maximum photochemical efficiency of photosystem II in the leaves of *Sedum cacuasicum* under shaded and lighted conditions.

As seen in the figure the quantity of the titratable acidity in *S. caucasicum* leaves decreased with increasing duration of the drought stress. Negative correlation is observed between the quantity of titratable acidity in the plants subjected to drought and relative water content in leaves. The value of lowering ΔH^+ is characteristic of CAM plants under severe water stress (Lee and Griffiths, 1987). CAM plant *S. dasyphyllum* shows, under controlled conditions, a decrease of malate accumulation as relative water content decreases. Recovery from water stress is fairly slow. Water potential quickly increases during rewatering and exceeds the original value after few days, suggesting a consumption of osmotic compounds during the water stress period (A. Fioretto et al., 1990).

PSII is known to be sensitive to the harmful environmental effects. The F_v/F_m ratio is a parameter that allows the detection of any damage to the photosystem II (PS II) and the possible existence of photoinhibition (Long et al. 1994). Diurnal changes in the photochemical activity of PS II depending on the environmental temperature and the intensity of solar radiation were measured in leaves of *S. cacuasicum* under outdoor conditions (under

shaded and lighted conditions) in the Absheron peninsula in July (Figure 3).

According to the obtained results the values of the photochemical efficiency of photosystem II (F_v/F_m) in the leaves of *Sedum cacuasicum* under shade condition were very close and almost unchanged. However, in plants grown under natural light these values decreased till afternoon with increasing light intensity and then remained stable. Previous values recovered with the decreasing light intensity by the evening. It suggests that the increasing light intensity causes damage to PSII, which leads to photoinhibition. But in accordance with the decreased light intensity and environmental temperature by the evening, this value became equal to the values obtained in the morning. This confirms that recovery process is faster in CAM plants and their plasticity is at the high level.

Phase III of CAM takes place under closed stomata and high irradiance, and PSII can become over-energized (Niewiadomska & Borland, 2008; Lüttge, 2010). Under such conditions, plants evoke photoprotection by non-radiative excess energy dissipation via xanthophyll cycle (Horton & Ruban, 2005; Murchie & Niyogi, 2011). Uptake, and diurnal

acidity changes sustained by nocturnal re-fixation of respiratory CO₂ (Sayed 2001b; Lüttge, 2010). Comparison of chlorophyll fluorescence parameters measured in *E. triaculeata* during wet and dry seasons indicated reduction of Fv/Fm and ΦPSII denoting reduced efficiency of PSII antenna and PSII quantum yield, respectively (Baker, 2008). Similar reduction of PSII activity manifested by reduction of Fv/Fm and PSII was reported for other CAM plants under stress conditions (Mattos et al., 1999).

Determination of titratable acidity both natural and controlled conditions revealed diurnal oscillation of acidification-deacidification cycles reflecting operation of obligate CAM. Nocturnal CO₂ uptake and daytime stomatal closure of CAM implies avoidance of gas exchange when environmental conditions favour transpirational water loss and improved plant water economy (Lüttge, 2008). The obtained results confirm that *Sedum caucasicum* is an obligate CAM plant.

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Yeni CAM Növü *Sedum caucasicum* Bitkisinin Yarpaqlarında Sutkalıq Titirlənən Turşuluğun Dəyişməsi

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Bitkilərdə fotosintetik yolların öyrənilməsi müasir bitki örtüsünün qiymətləndirilməsi və baş verə biləcək dəyişikliklərin proqnozlaşdırılması üçün çox əhəmiyyətlidir. Krassuliyasiya turşuları metabolizmi (CAM), gecə ərzində CO₂ qazının fiksasiyasına əsaslanan və sudan effektiv istifadənin artması ilə səciyyələnən karbonun fotosintetik assimilyasiya yollarından biridir. *Sedum* cinsinin növləri arasında CAM fotosintezin müxtəlif formalarına təsadüf olunur. Buraya C₃, CAM və fakültativ CAM növlər daxildir. Qafqaz üçün endemik olan *Sedum caucasicum* bitkisiində sutkalıq titirlənən turşuluğun dəyişməsi həm təbii şəraitdə, həm də süni iqlim şəraitində bitən bitkilərdə oxşar olmuşdur. Alınan nəticələr belə guman etməyə əsas verir ki, *S.caucasicum* bitkisiində CO₂ qazının fiksasiyası fotosintezin CAM yolu ilə baş verir.

Açar sözlər: CAM fotosintez, *Sedum caucasicum*, titirlənən turşuluq, fotokimyəvi effektivlik

Суточные Изменения Титруемой Кислотности В Листьях Нового CAM Вида *Sedum caucasicum*

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Изучение путей фотосинтеза в растениях очень важно для оценки современной растительности и для прогнозирования климатических изменений. Метаболизм по типу толстянковых (CAM) - один из способов поглощения углерода, который основан на фотосинтетической ассимиляции углерода в течение ночи и характеризуется увеличением эффективного использования воды. В роде *Sedum* обнаружена большая вариация форм CAM фотосинтеза, таких как: C₃, CAM и CAM-индуцируемые виды при водном или солевом стрессе. У вида *Sedum caucasicum*, который считается эндемичным для Кавказа, суточное изменение кислотности было аналогичным как для растений, выращенных в естественных условиях, так и для растений, выращенных в условиях искусственного климата. Результаты показали, что *S.caucasicum* является облигатным CAM растением.

Ключевые слова: CAM фотосинтез, *Sedum caucasicum*, титруемая кислотность, фотохимическая эффективность

Isoenzyme Spectrum, Localization and Some Physicochemical Properties of NAD-Malate Dehydrogenase in Amaranth Leaves Under Drought

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Malate dehydrogenase (MDH) plays a crucial role in energy and cell metabolism. Activity, subcellular distribution, isoenzyme spectrum and kinetic properties of cytosolic (cMDH) and mitochondrial malate dehydrogenases (mMDH) have been studied in the phases of pre-anthesis, anthesis and grain ripening. Subcellular localization, isoenzyme spectrum and kinetic properties of NAD-malate dehydrogenase (l-malate-NAD-oxidoreductase, NAD-MDH, EC 1.1.1.37) were studied in subcellular fractions (SCFs) of assimilating tissues of *Amaranthus cruentus* L. leaves during pre-anthesis, anthesis and grain ripening phases. NAD-MDH was found to have a wide isoenzyme spectrum in amaranth leaves, which changed depending on the number of isoenzymes, type of tissues and SCFs. Main part of the enzyme general activity was localized in cytosolic and mitochondrial fractions of both tissues. V_{\max} (OAA) value of the reaction catalyzed by cNAD-MDH in mesophyll cells (MC) were 2-times higher compared with V_{\max} (OAA) of the reaction catalyzed by mNAD-MDH. Whereas, in bundle sheath cells (BSC) V_{\max} (OAA) for mNAD-MDH was 3-4-times higher than the same parameter of the enzyme in MC. The enzyme isoforms had a wide range of pH optimum and were tolerant to temperature. Depending on SCFs the enzyme had higher sensitivity to the substrate oxaloacetate (OAA) and lower sensitivity to malate. The kinetics of the enzyme follows Michaelis-Menten plot and this enzyme is not allosteric. The enzyme activity is strongly regulated by substrates, mono and bivalent ions. Inhibitory effect of ATP to the all isoforms even more increased under drought.

Keywords: *Amaranth; drought; NAD-MDH; isoforms; localization; kinetics; tolerance*

INTRODUCTION

NAD-malate dehydrogenase (MDH; EC 1.1.1.37) catalyzes the reversible reaction of OAA conversion into malate. This reaction occurs due to the oxidation and reduction of NAD coenzyme (Minarik et al., 2002). Most eukaryotes have cytosolic and mitochondrial types of MDH (Sasaki et al., 2014). Citric acid cycle is a central oxidative pathway in aerobic procaryotes and eucaryotes. In this cycle the reversible reaction of malate conversion into OAA is catalyzed by NAD and NADP-MDH (Takahashi-Iñiguez et al., 2016). The enzymes of plant MDH system are considered as dynamic balance of proteins capable of responding to the organism requirements, physiological state, and environmental changes (Pinheiro et al., 1991).

MDH occurs in all living organisms including archaeobacteria and mammals and in all subcellular organoids (mitochondria, glyoxisomes, peroxisomes, chloroplasts etc.) (Selinski et al., 2014)

All MDHs are multimeric enzymes consisting of 2 and 4 identical subunits (Minarik et al., 2002; Yashvant et al., 2013). Plant mNAD-MDH is assumed to function in several directions. It oxidizes malate to OAA-the final product of the classical Krebs cycle. mNAD-MDH participates

not only in the oxidation of NADH in the Krebs cycle, but also in the metabolism of reducing equivalents among metabolic pathways in the various cell compartments (Minarik et al., 2002; Tomaz et al., 2010). According to some authors, MDH isoforms are related not only to respiration (Tomaz et al., 2010), but also to the β -oxidation of fatty acids, grain germination and stress tolerance (Pracharoenwattana et al., 2007; Cousins et al., 2007; Wang et al., 2014).

Thus, having an important and complex role, NAD-MDH participates in carbon and energy distribution in higher plants, which arises new ideas on molecular mechanisms connecting respiration, photosynthesis and photorespiration (Cousins et al. 2007; Tomaz et al., 2010).

The main purpose of our research was to study the activity, isoenzyme spectrum, localization and some physicochemical and kinetic properties of NAD-MDH in SCFs (cytoplasm, mitochondria) of assimilating tissues (AT) of amaranth leaves during active phases of plant development under drought.

MATERIALS AND METHODS

NAD-ME type C_4 plant *Amaranthus cruentus* L. cultivated under natural conditions was used as

the research object. Soil drought was imposed on the 30th day of the development by ceasing watering, while control plants were watered till the end of the vegetation.

To study subcellular distribution of the enzyme, MC and BSC were isolated using the mechanical method, purified and SCFs were derived. For this purpose the method developed by Edwards and Gardestrom (Gardestrom, Edwards, 1983) for the maize leaves was modified according to the studied object (Guliev et al., 2003).

Enzyme preparation: Leaves were detached from stems, washed with distilled water, dried on filter paper, cut into small pieces and homogenized for 2 min at +4°C in 100 mM Tris-HCl buffer (pH 8.0), containing 20 mM MgCl₂·6H₂O, 1 mM EDTA, 5 mM DTT, 1% Triton X-100 and 0.5% PVP-25 (1:5, m/V). The obtained homogenate was filtered through 2-fold capron cloth and centrifuged for 5 min at 1000g and then for 15 min at 5000g to remove nucleus and tissue residues. After removing the precipitate, supernatant was used for the determination of the enzyme activity.

NAD-MDH activity: NAD-MDH activity was determined spectrophotometrically (Ultrospec 3300 pro, Amersham, USA) (Scheibe, Stitt, 1998). The reaction medium for the OAA reduction was Tris-HCl buffer (pH 8.0), containing 1mM OAA, 10 mg/ml BSA, 10 mM MgCl₂·6H₂O, 0.15 mM NAD·H and 5-10 µl enzymatic preparation. The reaction started by adding 1 mM OAA into the reaction medium. The reaction medium for the malate oxidation was 100 mM Tris-HCl buffer (pH 9.0), containing 30 mM malate and 0.2 mM NAD. The enzyme activity was determined spectrophotometrically based on the decline in optic density due to the NAD·H expenditure for 1 min. Extinction coefficient for NADH and NADPH was 6.22 mM·cm⁻¹ at 340 nm.

Native gel electrophoresis was performed on the polyacrylamide gel (PAAG) (Sigma, USA, Mo 63178, Model E-4266) at +4°C (Davis, 1964).

Specific detection of NAD-MDH: detection of NAD-MDH isoforms on the electrophoretic gels was performed in a specific medium using the tetrazole method (Fieldes, 1992). To detect NAD-MDH isoforms using gel-electrophoresis, gels were incubated in a fresh reaction mixture (100 ml 0.1 M Tris-HCl buffer, pH 8.0, containing 0.05 M malate, 0.02 M NAD⁺, 0.01 M nitro-tetrazolium blue, 0.01M phenasine metasulfate) prior to fixation at 37°C for 35-40 min.

Molecular weight of NAD-MDH was determined using gel-electrophoresis (Laemmli, 1970). Protein markers such as β-galactosidase (116 kDa), bovine serum albumin (BSA) (66.2 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35 kDa), restriction endonuclease BSP981 (25 kDa),

α-lactate globulin (18.4 kDa) and lysozyme (14.4 kDa) were used for the determination.

Total protein was determined according to the Sedmak, Grossberg method. BSA was used for the construction of the calibration curve. (Sedmak and Grossberg, 1997).

Statistical analysis: the data presented in the tables and figures are the mean values of at least three biological and mathematical replicates.

RESULTS AND DISCUSSION

C₄ plants are known to be more tolerant to extreme environmental factors compared to C₃ plants. Activity, localization and isoenzyme content of NAD-MDH in amaranth leaves were studied to clarify the role of the enzyme in the creation of biochemical mechanisms of plant tolerance. The obtained results are presented in Figures 1-2 and in Table 1.

Table 1. Subcellular distribution of NAD-MDH activity in amaranth leaves under soil drought conditions during anthesis (A - specific activity, U/mg protein)

Object, Variant	NAD-MDH activity			
	Cytosol		Mitochondria	
	A	%	A	%
Anthesis				
C	56.6±1.68	28.0	125.5±3.2	61.6
MC D	46.3±1.43	23.6	123.4±4.1	63.1
K	79.5±1.71	31.8	152.0±4.4	60.6
BSC D	51.1±0.99	25.1	140.0±3.8	69.0

Note: MC - mesophyll cells, BSC - bundle sheath cells, C - control, D - drought.

As seen in Figure 1 in the pre-anthesis phase NAD-MDH activities were similar in drought and control variants in MC and BSC of cytosolic fraction at the beginning of drought. However, in the anthesis and grain ripening phases the enzyme activity increased in both variants and this increase was more in drought-exposed plants than in control plants.

The activity of mesophyll enzyme in MC and BSC (Figure 1B) was higher in all phases compared with cytosolic enzyme (Figure 1A). The enzyme activity increased in the drought-exposed plants and decreased only at the end of the vegetation, though this decrease occurred slower. Thus, the enzyme activity in the mitochondrial fraction was ~2.5 times higher in the pre-anthesis phase and ~2 times higher in the anthesis phase compared with the cytosolic fraction.

Electrophoresis revealed that NAD-MDH of amaranth leaves had a wide range of isoenzyme spectrum and isoform changes occurred in the isoenzyme spectrum under drought. Thus, five

isoforms of the enzyme with molecular weights of 58, 63, 68, 72 and 77 kDa were detected in SCFs of amaranth leaves, in the initial period of stress during active pre-anthesis phase of ontogenesis (Figure 2A). The distribution of these isoforms in SCFs was as follows: 58, 63, 68 and 77 kDa isoforms were localized in MC, 63 and 72 kDa isoforms in cytosol of BSC, 63 kDa isoform in chloroplasts of MC and BSC, 63, 68 and 77 kDa isoforms in mitochondrial fractions of MC and BSC.

No change was observed in cNAD-MDH of MC during the pre-anthesis and anthesis phases in control plants. However, 63 and 68 kDa isoforms disappeared under drought. During the phase of grain ripening 63, 68 and 77 kDa (with the exception of 58kDa isoform) isoforms of cNAD-MDH disappeared in MC of control plants. An inductive, 72 kDa isoform emerged under drought in addition to 58 and 77 kDa isoforms. All the isoforms disappeared at the end of vegetation and only 58 kDa isoform remained in cytosolic fraction of MC of control plants.

Different isoenzyme change patterns were observed in MC and BSC. As seen in the Figure in the cytosolic fraction of BSC of both control and drought variants 2 constitutive isoforms of the enzyme (63 and 72 kDa) were present in the pre-anthesis phase. Isoenzyme spectrum changed in the anthesis phase, 58 and 77 kDa isoforms emerged in control, while 58 kDa inductive isoform emerged in the drought-exposed variant. However, in the grain ripening phase only 58kDa isoform emerged in

control, while 68 kDa inductive isoform emerged along with 58 kDa isoform in plants exposed to drought. The intensity of this isoform sharply declined to the end of vegetation (Figure 2).

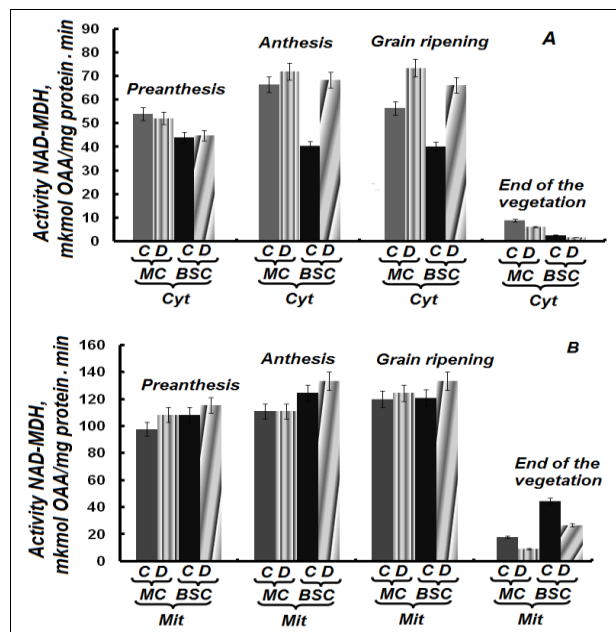


Figure 1. Dynamics of changes in NAD-MDH activity in subcellular fractions of MC and BSC in amaranth leaves under drought. A-cytosol, B-mitochondria, MC-mesophyll cells, BSC-bundle sheath cells, C-control, D-drought, OAA-oxaloacetate.

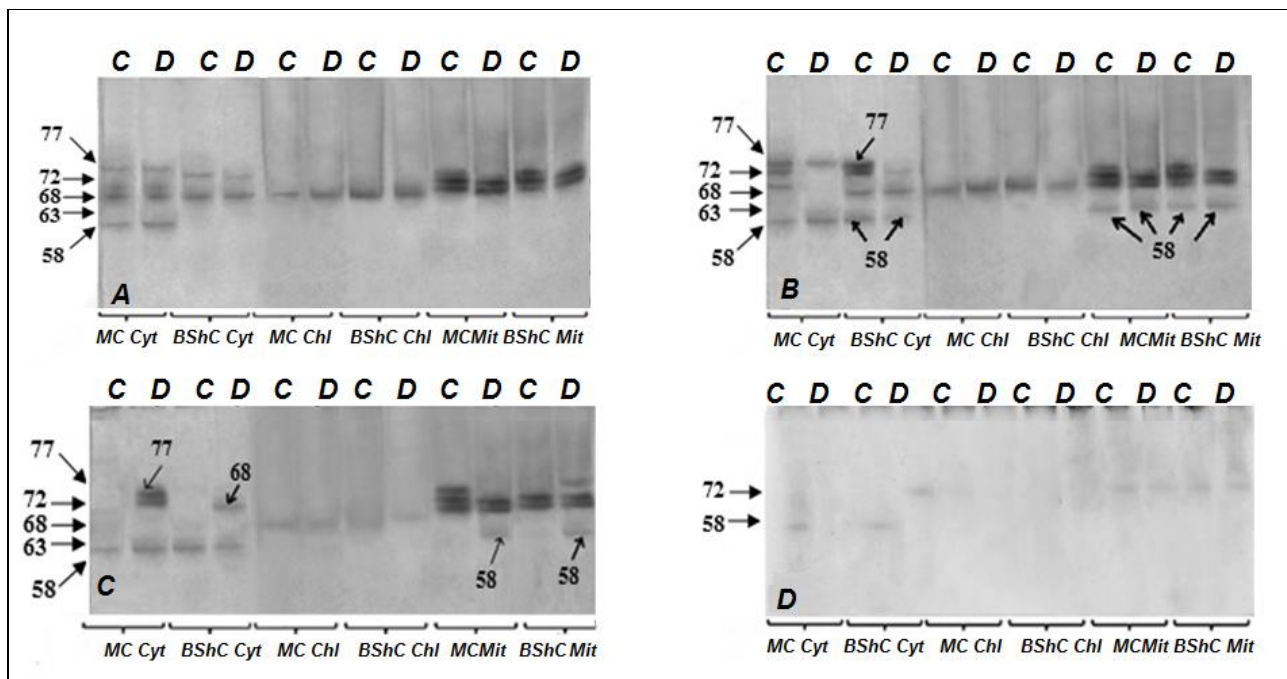


Figure 2. Determination of NAD-MDH isoforms in subcellular fractions of MC and BSC in drought-exposed amaranth leaves using gel-electrophoresis. A-pre-anthesis, B-anthesis, C-grain ripening and D-the end of vegetation stages. Cyt-sitoplasma, Chl-chlorophyll, Mit-mitochondria, C-control, D-drought.

Table 2. Some kinetic parameters of the reaction catalyzed by NAD-MDH in subcellular fractions of MC and BSC of the leaves of amaranth exposed to soil drought in the anthesis phase.

Object,	Vari- ant	OAA (0.1-10 mM)		Malate (1-50 mM)		MgCl ₂ (1-50 mM)		ATP (0.1-3 mM)		
		K _m	V _{max}	K _m	V _{max}	K _m	V _{max}	K _i	V _{max}	
Anthesis										
MC	Cyt	C	0.70	23.6	0.06	71.4	3.9	47.6	1.50	100.0
		D	0.53	43.4	0.05	333.0	3.9	100.0	2.10	20.0
	Mit	C	0.40	10.0	0.72	50.0	2.8	192.0	1.30	125.0
		D	0.30	50.0	0.40	111.0	4.0	240.0	1.20	100.0
BSC	Cyt	C	0.50	40.0	0.05	100.0	4.6	45.5	1.00	83.3
		D	0.63	100.0	0.04	250.0	5.0	100.0	2.30	33.3
	Mit	C	0.65	25.0	0.19	50.0	4.9	10.0	1.20	100.0
		D	0.51	50.0	0.23	100.0	6.0	25.0	1.03	66.7

Note: MC - mesophyll cells, BSC - bundle sheath cells, C - control, D - drought, Cyt - cytosol, Mit - mitochondria, OAA - oxaloacetate. V_{max} - mM/mg protein.

Note: MC - mesophyll cells, BSC - bundle sheath cells, C - control, D - drought, Cyt - cytosol, Mit - mitochondria, OAA - oxaloacetate, V_{max} - mM/mg protein.

Three NAD-MDH isoforms with molecular weights of 63, 68 and 77 kDa were detected in mitochondrial fractions of both tissues in pre-anthesis and anthesis periods in control variants. In the pre-anthesis phase 77 kDa isoform disappeared in both tissues under drought conditions and 58 kDa inductive isoform emerged in both tissues of both variants in the anthesis phase.

In the grain ripening phase of control plants 58 kDa inductive isoform disappeared in MC and BSC of the mitochondrial fraction, while 58 kDa isoform remained and 72 kDa isoform emerged in BSC of the mitochondrial fraction under drought (Figure 2, Table 2) and only traces of 68 kDa isoform were detected. The differences observed in the NAD-MDH isoenzyme spectra of SCFs of assimilating tissues under drought are possibly related to metabolic properties of these tissues. Thus, one of the alternative ways of the plant protection against CO₂ deficiency due to the stomatal closure under water stress is the acceleration of synthesis rate of C₄ acids at the expense of MDHs. An increase in mNAD-MDH activity enhances the synthesis of C₄ acids. An enhancement in the synthesis of C₄ acids activates carbon concentrating mechanism upon stomatal closure and reduction of gas-exchange, and as a result Calvin cycle is provided with CO₂ (Wang et al., 2014).

The obtained results confirmed that complex anatomic structure of C₄ plant leaves obtained through the course of evolution resulted in the formation of photosynthesizing tissues and complexity in the function of the enzymatic systems and in the isoenzyme content due to stress.

Being very mobile NAD-MDH isoforms in SCFs of amaranth leaves can change depending on the phases of plant development and drought effects, and an isoform constitutive for one fraction may be inductive for another under stress. cNAD-MDH is less studied than mNAD-MDH. cNAD-MDH participates in some shuttle mechanisms and in the metabolism of substrates and reducing equivalents

between cytoplasm and other cell organoids (Yu, Qing-Hu, 2004). Temperature sensitivity is one of the important properties of the enzymes. Temperature plays an important role in the regulation of activities of enzyme systems, biochemical conversions, formation of the balance between the enzyme molecule and enzyme-substrate complex (Cook, Cleland, 2007; Cornish-Bowden, 2012). Temperature influences on the enzyme stability, the rate of the destruction of the enzyme-substrate complex, enzyme-substrate affinity, enzyme affinity for activator and inhibitor (Cornish-Bowden, 2012).

The results obtained for SCFs of assimilating tissues showed that gradual rise in temperature till 45°C resulted in the increase of the enzyme activity, maximum rate of the reaction was gained at 45-55°C, and at 70-80°C the enzyme activity was minimum, due to the denaturation of the enzyme structure. Optimum temperature range for NAD-MDH from the leaves of monocot C₃-plant was 45-50°C. According to the reports optimum temperature range for MDHs from various resources was 30-60°C (Pinheiro et al., 1991).

The dependence between the rate of the direct and reverse reactions catalyzed by NAD-MDH and concentrations of substrates - OAA and malate was studied and the results are presented in Figures 3- 4 and Table 2. The isoforms localized in SCFs of amaranth assimilating tissues catalyzed the reaction rate stronger at 1.0 mM concentration of OAA under normal conditions. The NAD-MDH activity at 1.0 mM concentration of OAA increased by 20% under drought. As seen in Table and Figures, K_m(OAA) and V_{max} (OAA) values for sNAD-MDH and mNAD-MDH of mesophyll cells increased similarly in control and drought-exposed plants.

K_m (OAA) of cNAD-MDH in BSC decreased under drought, whereas V_{max} (OAA) increased. However, for mNAD-MDH of BSC the values of both K_m (OAA) and V_{max} (OAA) increased under drought (Figure 3, Table 2).

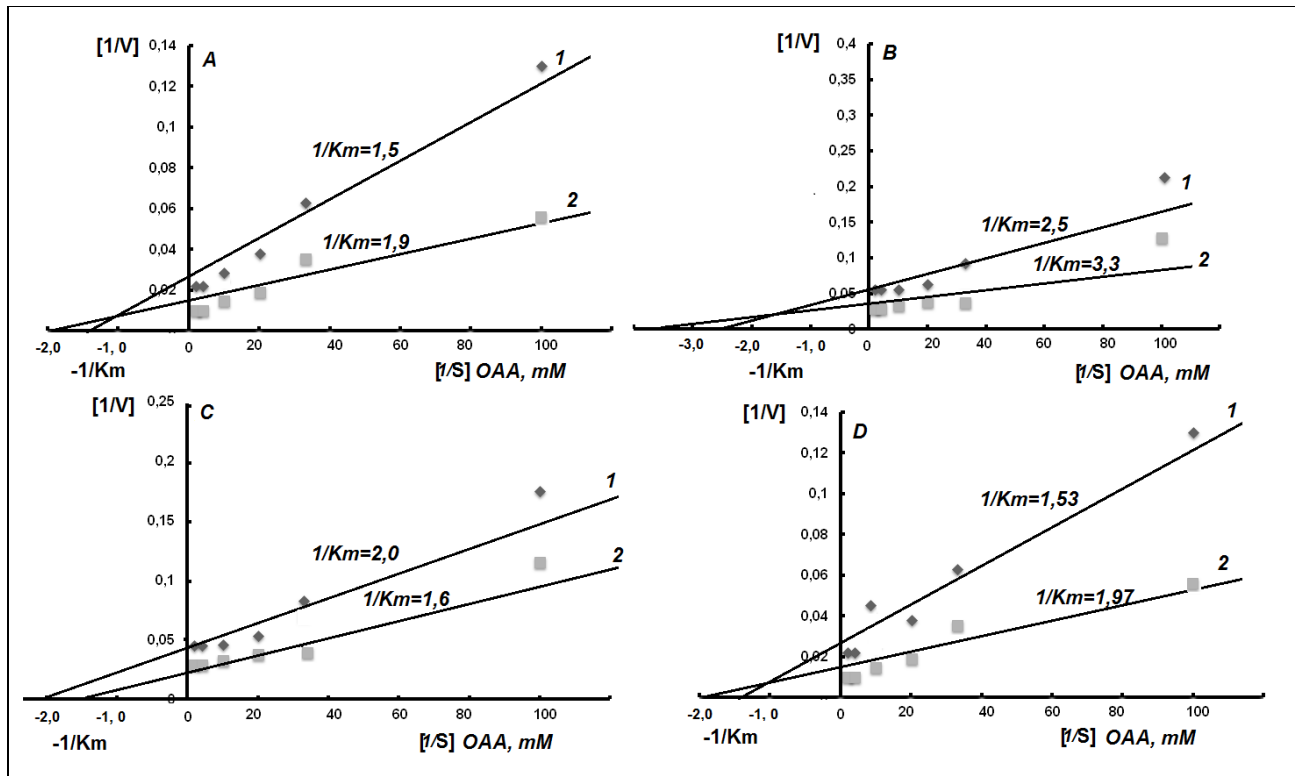


Figure 3. Kinetics of the changes of OAA reduction reaction depending on the substrate concentrations in various subcellular fractions of MC and BSC during the anthesis phase.

1 - Control, 2 - Drought. A - MC, cytosol, B - BSC, mitochondria, C - BSC, cytosol, D - BSC, mitochondria.

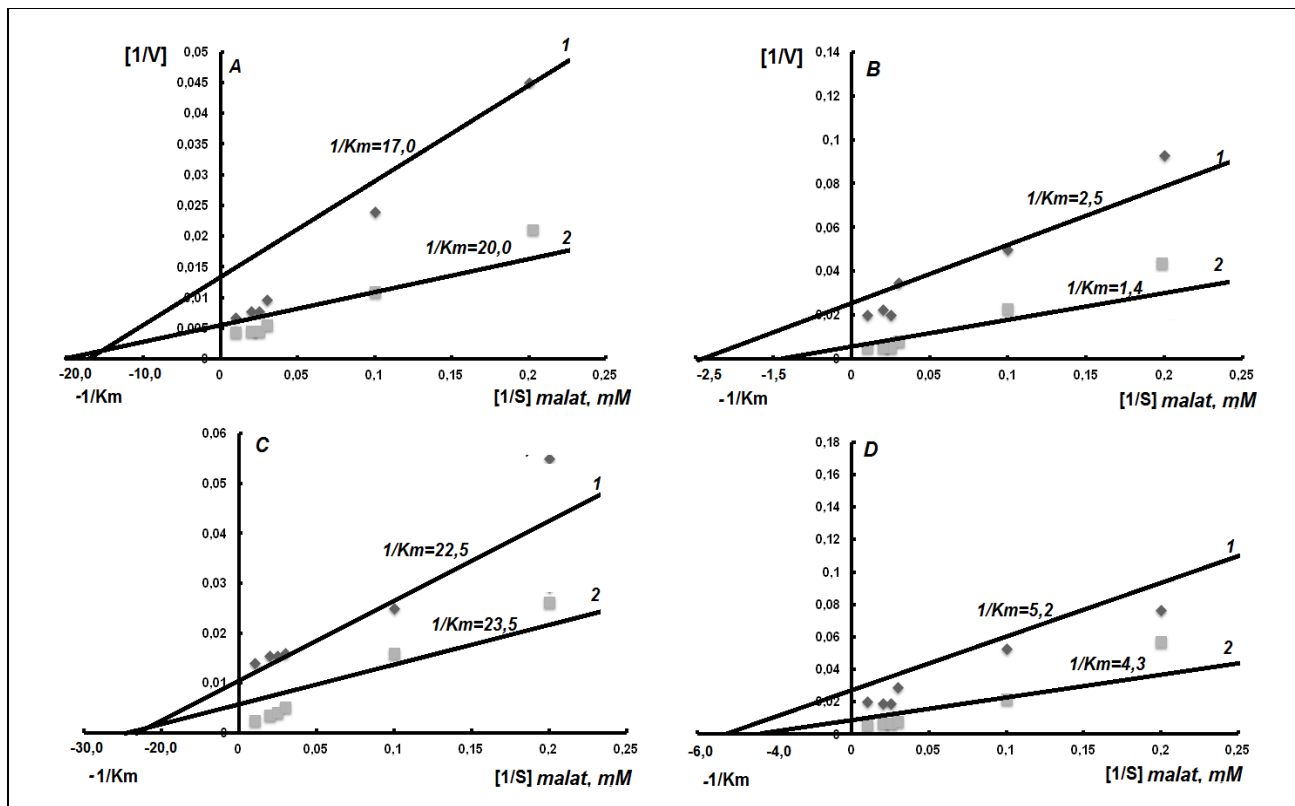


Figure 4. Kinetics of the changes of the reaction of malate reduction depending on the substrate concentrations in various subcellular fractions of MC and BSC during the anthesis phase.

1 - Control, 2 - Drought. A - MC, cytosol, B - BSC, mitochondria, C - BSC, cytosol, D - BSC, mitochondria.

The analysis of the results shows that K_m (OAA) values for the reaction catalyzed by NAD-MDH in amaranth leaves (1.2-2.1 mM) were less than those obtained for the same values in flag leaves of wheat genotypes. V_{max} values in BSC of amaranth leaves are 3-5 times higher compared with V_{max} of the same reaction catalyzed in SCFs of mesophyll cells of wheat flag leaves. In amaranth V_{max} was always higher in BSC than in MC and this difference increased under drought. In the cytosolic fraction of MC V_{max} was ~2 times higher than in the mitochondrial fraction. Although K_m (OAA) and V_{max} (OAA) values were almost the same in the control and drought variants of cytosolic fractions of MC and BSC, V_{max} (OA) in the mitochondrial fraction of BSC was ~3-4 times higher compared with that in the mitochondrial fraction of MC.

The obtained results show that MDH is more sensitive to OAA, than to malate and these data are consistent with the results of other researchers (Figure 3, 4; Table 2). It was reported that low sensitivity of the enzyme to malate is related to the conformational changes of malate during its catalytic conversion (Сатар и др., 2010). The comparison of K_m values shows that cNAD-MDH is less sensitive to malate than to OAA. K_m values depended on plant species, type of the metabolism, the function performed in the cell and growth conditions (Figure 3, 4; Table 2). Analysis of the enzyme kinetic properties according to Lineweaver-

Burk plots showed that during the enzyme inhibition K_m remained unchanged, while V_{max} decreased. As seen in the figure in SCFs of amaranth leaves 20-40 mM concentrations of malate caused an increase in the enzyme activity (Figure 3, 4; Table 2).

In direct and reverse reactions NAD-MDH exists in two conformation states differing in kinetic parameters. As malate oxidation is accompanied by overcoming additional energetic barrier and significant conformation changes in the structure of molecule, the reaction does not proceed with a stable rate. The OAA conversion reaction is not related to conformational changes occurring in the enzyme structure.

The differences in the kinetics of direct and reverse reactions may be due to the physiological functions, which NAD-MDH performs in SCFs of amaranth leaf cells.

Activators and inhibitors play an important role in the regulation of the enzyme activity. Activators facilitate substrate binding in the active center, formation of the enzyme-substrate complex, formation and stabilization of three-dimensional structure. Table 3 shows kinetics of the activating effects of Mg^{2+} ions on NAD-MDH isoforms in SCFs of amaranth leaves. As seen in the Table and Figure low concentrations of Mg^{2+} ions stabilize, while high concentrations inhibit MDH isoforms (Figure 5, Table 3).

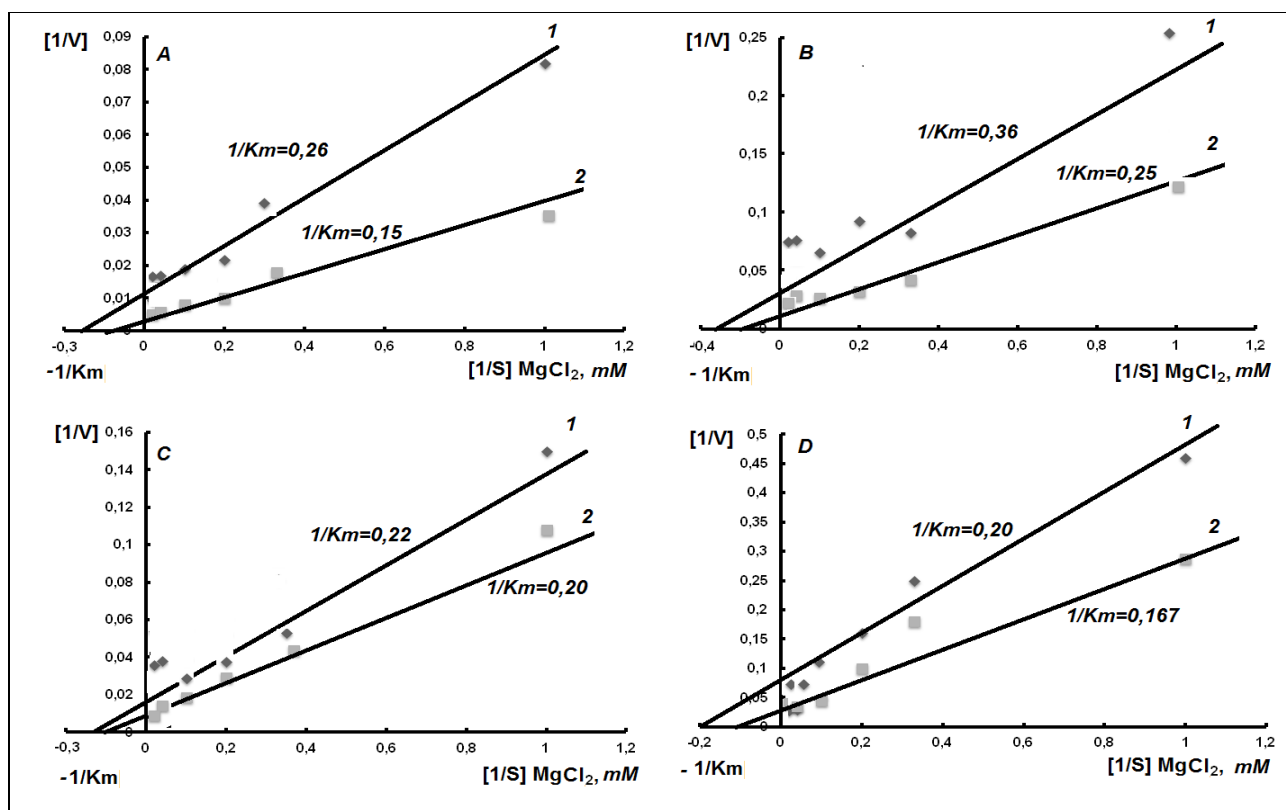


Figure 5. Kinetics of the NAD-MDH activation by Mg^{2+} in anthesis phase of amaranth plants exposed to soil-drought. 1 – control, 2 - drought, A - cytosol, B – mitochondria.

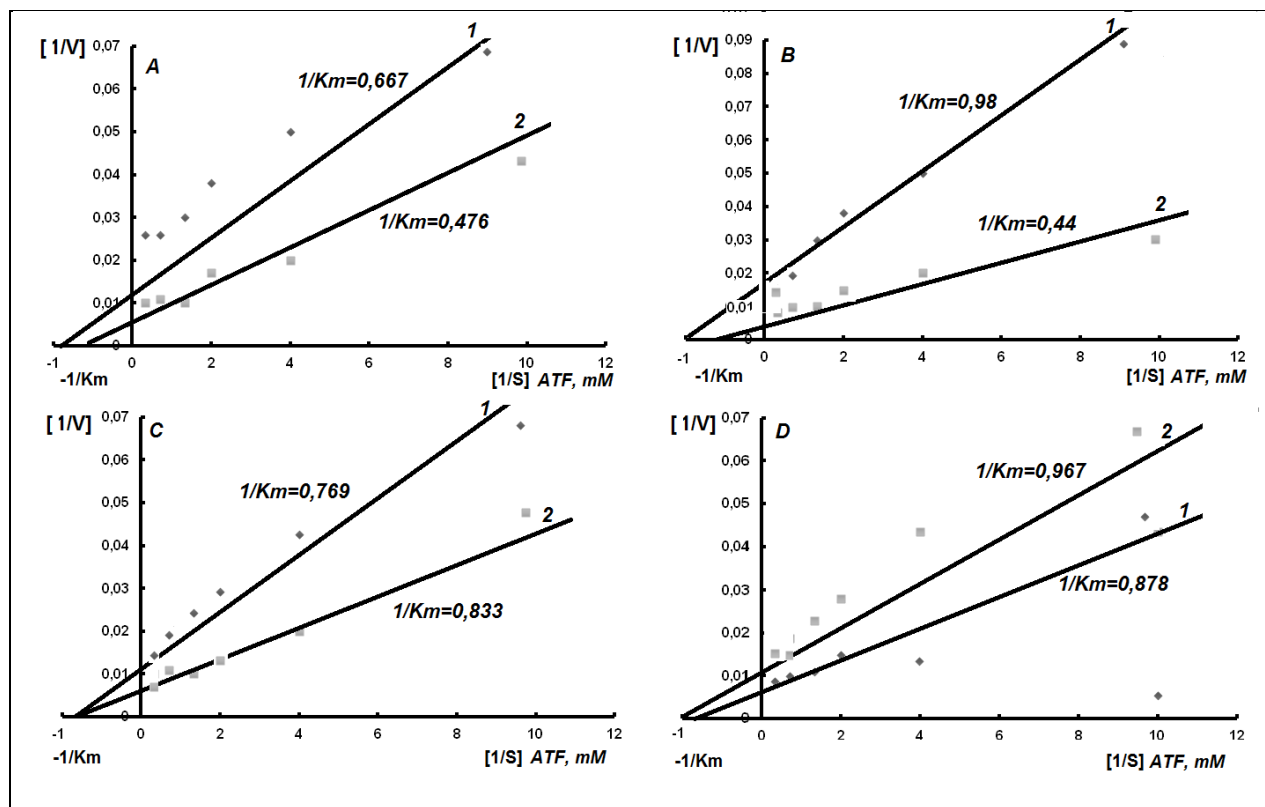


Figure 6. Kinetics of the NAD-MDH inhibition by ATP in anthesis phase of amaranth plants exposed to soil-drought. 1 – control, 2 - drought, A - cytosol, B – mitochondria.

According to some authors Mg^{2+} ions act as a competitive inhibitor of the MDH molecule [11]. Activating effect of Mg^{2+} ions on MDH isoenzymes can be related to the interaction of substrates with metal ions, which results in stabilization. K_m and V_{max} values obtained in the experiments performed with subcellular fractions from leaves of well-watered and stressed plants revealed that this ion was not a specific cofactor for the MDH isoforms.

ATP was found to be the efficient inhibitor of the enzymes of plant and animal origin which contradicts the previous reports. Results of the experiments showed that among MDH isoforms mMDH was more sensitive to ATP effects.

MDH is suggested to be one of the crucial links in the concerted action of metabolic pathways such as catabolism (Krebs cycle) and anabolism (gluconeogenesis). This feature is related to the fact that, mMDH is one of the main elements of the regulatory site between malate and citrate in the Krebs cycle. The mNAD-MDH activity is strongly controlled by the $NAD^+/NADH$ and ATP/ADP ratios (Amthor, 2010).

Inhibitory effect of various ATP concentrations is presented in Figure 6 and Table 2. It was found that NAD-MDH activity of amaranth leaves sharply declined at 3 mM concentration of ATP.

As seen in the Table and Figure maximum rate of the reaction catalyzed by NAD-MDH (V_{max}) in MC decreased 5 and 0.25 times in cytosolic and

mitochondrial fractions of amaranth leaves under drought, while 2.5 and 1.5 –fold decreases were detected in BSC, respectively. The obtained results confirmed that ATP inhibited cMDH localized in MC more than the enzyme localized in BSC, probably due to the subcellular localization of cMDH.

CONCLUSION

It was concluded that isoenzyme spectrum of amaranth NAD-MDH changed depending on the type of assimilating tissues, subcellular fraction and effects of drought stress. This change was more observed in cytosolic and mitochondrial fractions.

The disappearance of some constitutive isoforms of NAD-MDH and emerging of some inductive isoforms can be considered as a component of adaptive mechanism of plants against drought, which neutralizes stress effects and protects photosynthesis and CO_2 metabolism. This is also confirmed by the synchronous changes in the enzyme activity and some kinetic parameters. Thus, qualitative and quantitative changes occurring in the activity, isoenzyme spectrum, physicochemical and kinetic properties of the enzyme in assimilating tissues of amaranth leaves under the influence of drought and climatic factors show that NAD-MDH is a labile and adaptive enzyme.

ACKNOWLEDGEMENT

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Quraqlıqda Amarant Yarpaqları NAD-Malatdehidrogenazasının İzoferment Spektri, Lokalizasiyası və Bəzi Fiziki-Kimyəvi Xassələrinin Tədqiqi

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Malatdehidrogenaza (MDH) enerji və hüceyrə metabolizmində mühüm rol oynayır. Bu məqalədə amarant yarpaqlarında sitozol (sMDH) və mitoxondri (mMDH) malatdehidrogenazalarının aktivliyi, subhüceyrə paylanması, izoferment spektri və kinetik xassələrinin quraqlığın təsirindən dəyişməsi bitkinin inkişafının çiçəkləmə öndü, çiçəkləmə, toxum yetişmə fazalarında tədqiq olunmuşdur. *Amaranthus cruentus* L. yarpaqlarının assimilyasiyaedici toxumalarının subhüceyrə fraksiyalarında (SHF) bitkinin inkişafının çiçəkləməöndü, çiçəkləmə, toxum yetişmə fazalarında quraqlığın təsirindən NAD-malatdehidrogenaza (I-malat-NAD-oksireduktaza, NAD-MDH, EC 1.1.1.37) fermentinin aktivliyinin subhüceyrə paylanması, izoferment spektri və kinetik xassələri tədqiq olunmuşdur. Müəyyən olunmuşdur ki, amarant yarpaqlarında NAD-MDH geniş izoferment spektrinə malikdir və izoformaların sayı stres, toxuma növü və SHF-dan asılı olaraq dəyişir. Fermentin ümumi aktivliyinin əsas hissəsi hər 2 toxumanın sitozol və mitoxondri fraksiyalarına lokalizə olunmuşdur. MH-nin sNAD-MDH-sının kataliz etdiyi reaksiyanın V_{\max} (OA) qiyməti mNAD-MDH-ya nisbətən 2 dəfə, ÖTH-nin mNAD-MDH-sının V_{\max} (OA) qiyməti isə MH-nin mNAD-MDH müvafiq göstəricisindən 3-4 dəfə yüksək olmuşdur. Fermentin izoformaları geniş pH optimumuna malik olub temperatura qarşı davamlıdırlar. Ferment SHF-dan asılı olaraq öz substratı olan oksalasetata (OA) qarşı yüksək, malata qarşı isə aşağı həssaslığa malikdir. NAD-MDH reaksiyası ümumilikdə Mixaelis-Menten qanunauyğunluğuna tabe olub substrata qarşı heç bir allosteriklik göstərmir. Fermentin aktivliyi aralıq substratlar, bir və iki valentli ionlarla ciddi tənzim olunur. ATF-in fermentin bütün izoformalarına inhibirləşdirici təsiri quraqlığın təsirindən daha da artır.

Açar sözlər: Amarant, quraqlıq, NAD-MDH, izoforma, lokalizasiya, kinetika, tolerantlıq

Изоферментный Спектр, Локализация и Некоторые Физико-Химические Свойства NAD-Малатдегидрогеназы в Листьях Амаранта При Засухе

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Малат дегидрогеназа (MDH) играет основную роль в энергетическом и клеточном метаболизме. Активность, субклеточное распределение, изoferментный спектр и кинетические свойства цитозольной (цMDH) и митохондриальной малатдегидрогеназы (mMDH) изучались перед цветением, в фазах цветения и созревания зерна. Субклеточная локализация, изoferментный спектр и кинетические свойства НАД-малатдегидрогеназы (I-малат-НАД-оксидоредуктаза, НАД-МДГ, КФ 1.1.1.37) изучались в субклеточных фракциях (СКФ) ассимилирующих тканей листьев *Amaranthus cruentus* L. перед цветением, в фазах цветения и созревания зерна. Было обнаружено, что НАД-МДГ имеет широкий изoferментный спектр в листьях амаранта, который меняется в зависимости от количества изoferментов, типа тканей и СКФ. Основная часть общей активности фермента была локализована в цитозольных и митохондриальных фракциях обеих тканей. Значение V_{\max} (OA) реакции, катализируемой цитозольным ферментом в мезофильных клетках (МК), в 2 раза превышает значение V_{\max} (OA) реакции, катализируемой митохондриальным ферментом. Однако, в клетках обкладки (КО) V_{\max} (OA) для мНАД-МДГ в 3-4 раза превышает, V_{\max} (OA) в МК. Изоформы фермента обладают широким диапазоном оптимальных значений pH и устойчивы к температуре. В зависимости от СКФ фермент обладал высокой чувствительностью к оксоацетату (ОАА) и низкой чувствительностью к малату. НАД-МДГ подчиняется кинетике Михаэлиса-Ментена, и не является аллостеричным. Активность фермента строго регулируется субстратами, одно- и двухвалентными ионами. Ингибирующее действие АТФ на все изоформы увеличивается при засухе.

Ключевые слова: Амарант, засуха, НАД-МДГ, изоформы, локализация, кинетика, толерантность

Agar System (Agaroponics) for Modeling Abiotic and Biotic Effects on a Plant Organism (Manuals)

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A method of using agaroponics for studying the resistance and adaptability of wheat plants to stress effects is proposed. The possibility of modeling the increasing salt stress effect is shown.

Keywords: *Agaroponica, stressor, salt stress gradient, modeling, wheat*

The modern physiology of plants is characterized by a system-structural approach, and its correctness largely determines the success of further manipulations with experimental material.

The use of model systems at the level of a native plant is required in physiological, biochemical, biophysical and molecular-genetic studies.

The basis and success of the experimental approach depend on the stages of obtaining and cultivation of the initial material.

In natural habitats or in modeling natural conditions, plants are exposed to numerous stresses, and the response to them as a whole may differ from responses to individual effects or the sum of individual stresses. In this case, one stress can simulate the action of another (Kawa et al., 2016). At present, approaches based on the plant cultivation in the presence of certain factors, using water and sand cultures have been used. However, water and sand cultures, in spite of their simplicity, have a number of shortcomings, which ultimately have a negative impact not only on the interpretation of the results, they do not completely clarify the potential of the genotype in tests-experiments either (Besslavskaya et al., 1973; Grozdinskiy et al., 1973; Zhurbinskiy, 1968; Hyuit, 1960; Ivanova, 2004). For example, when using an aquatic culture, germination and cultivation of non-aquatic plants under the conditions of a given stressor effect occur in the presence of additional water stress, which, in its turn is aggravated by other types of stress. Water culture has shortcomings due to changes in metabolic processes during root uptake, difficulties in maintaining a certain concentration and reaction of the nutrient solution associated with simultaneous and uninterrupted supply of the root system with a solution of mineral elements, an increase in the toxicity of the stressor in aqueous solutions, the necessity for aeration and the complexity of determining the norm of requirement in the air supply, because of the low solubility of oxygen in water. In fact, the obligatory replacement of nutrient solutions, which

are necessary to maintain balance and equilibrium of nutrients, as an additional stage of manipulation, itself is a stress factor. This problem cannot be solved using the method of fluid solutions (Hyuit, 1960, Chernavina, 1978), since, ideally, additional equipment is required to monitor all parameters, and ultimately, all this together is a laborious process accompanied by an increasing stress.

One of the shortcomings of water cultures is a difficulty in providing sterility of nutrient solutions. This is important if the cultivated objects are needed for molecular genetic analysis. The use of various components of some antibiotics used for the inhibition of protein synthesis, which prevents bacterial contamination, can also significantly affect the results of the analysis.

Sand culture used in vegetation experiments is more preferable compared to aquatic culture, although it has the same drawbacks. The most important among them is the weak retention of nutrients on an inert substrate, their uneven distribution throughout the volume and, as a result, the weak buffer capacity of the sand mixture, the inability to observe and control the root system, the probability of the development of the bacterial factor (Zhurbitsky, 1968; Hyuit, 1960).

Existing methods of plant cultivation, such as hydroponics, agaroponics, chemoponics, aeroponics, are mainly intended for industrial cultivation of plants. For scientific research purposes, the most suitable method is the cultivation in agar medium. For the first time this method was developed to study the degree of adaptivity by modeling the increasing impact of salt stress. (Garagezov et al., 2012).

The presented research is devoted to the development of methodological approaches for determining the degree of resistance and adaptability of different varieties and forms of wheat to stressors with the aim of ranking them according to these characteristics. The plant cultivation method, proposed by us is based on the cultivation of plants in agar medium under sterile conditions (Fig. 1).

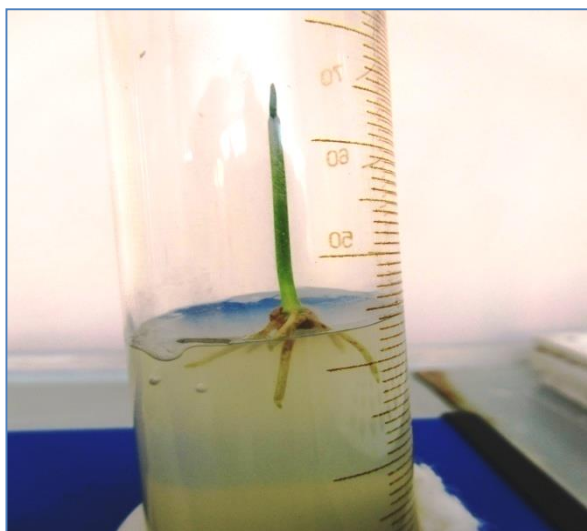


Figure 1. Cultivation of wheat plants in agar medium under salt stress in the initial period of development.

Agar medium contains the mineral nutrient medium Murashige and Skoog (M-S) (Murashige, 1962). As a basis for culture media, it is possible to use any nutrient media recommended for plant cultivation, based on the biological characteristics of the study object and the experimental tasks.

The choice of the MS medium is explained by the fact that of all media recommended for the cultivation of cereal plants under *in vitro* conditions, this medium has the most optimal and balanced composition of nutrients, and a sufficient buffer capacity throughout the entire cultivation period. The use of this medium allows also the coordination of results of *in vivo* experiments with studies of adaptive responses at the cellular level using *in vitro* model systems.

In addition, the presence of CaCl_2 in this medium makes possible modelling the effect of NaCl in studies on the resistance to NaCl , taking into account its toxicity. The concentration of CaCl_2 can vary depending on the content of the acting stressor, but the molar ratio of NaCl to CaCl_2 of 5: 1 is a required condition (Mamedova et al., 2010).

Composition and preparation of the mineral part of the nutrient medium:

1. Microelements: NH_4NO_3 - 1650 mg / l; KNO_3 - 1900 mg / l; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ - 370 mg / l; KH_2PO_4 - 170 mg / l. The macroelements are prepared in 10-fold concentration in 1L volume. 100 ml of stock solution per 1 liter of nutrient medium is used. It is possible to increase the multiplicity to 20, in this case 50 ml of the solution is used.

2. Microelements: H_3BO_3 - 6.2 mg / l; $\text{MnSO}_4 \times 4\text{H}_2\text{O}$ - 22.3 mg / l; $\text{ZnSO}_4 \times 4\text{H}_2\text{O}$ - 8.6 mg / l; KJ - 0.83 mg / l; $\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$ - 0.25 mg / l; $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ - 0.025 mg / l; $\text{CoCl}_2 \times 6\text{H}_2\text{O}$ - 0.025 mg / l.

3. CaCl_2 (440 mg / l) is added to the medium. It is convenient to prepare a concentrated solution (44g / 100ml H_2O) and to add 1ml of the solution to the medium.

4. Iron chelate: 557 mg of $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ is dissolved in cold water, 775 mg / l of Na_2EDTA is added, volume is brought to 100 ml and heated in a boiling water bath for 10-15 minutes. In the nutrient solution, 5 ml chelate is added per 1 liter. For the cultivation of wheat plants, it is recommended to use both the described above medium M-S, and the $\frac{1}{2}$ and $\frac{1}{4}$ parts of this medium (Murashige and Skoog, 1962).

5. After preparation of the liquid medium, its pH is adjusted to 5.6-5.8 with 0.1N NaOH or 0.1N HCl .

To prepare 0.6-0.8% solution half volume of molten agar was added into the half volume of nutrition medium. The medium is poured into culture vessels and autoclaved at 0.8 atm. for 30 minutes.

The volume of vessels used for growing plants is selected depending on the plant species and the duration of cultivation. We used graduated 100 ml cylinders, in which 50-60 ml medium was added, sufficient for the cultivation of wheat plants for 2 months. The proposed system acquires an exceptional advantage in studying the stressor effect with an increasing gradient of the acting factor, for example, secondary salinization.

Preparation of the agar medium with an increasing gradient of the active factor or with combined stressor effects

When the studies were performed under increasing stressor concentrations, a 2 or 3 component medium was also prepared as described above, but in this case, the medium containing the greatest stressor concentration was poured first into the culture vessel. After autoclaving and solidification of the medium, the remaining one-time constituents pre-autoclaved in separate volumes and cooled to about 40°C were added to the vessels with the medium under aseptic conditions. Fig. 2 shows the variants of the modeling scheme with the gradient of the increasing stress factor.

Taking into account the possibility of slight natural diffusion of NaCl , under an increasing gradient of the active factor, conditions are created for the smooth transition of growing roots from one concentration of NaCl in agar to another. This is a positive side of the proposed method, since it excludes a sharp change in the strength of the stress effect as an additional factor. Depending on the tasks of the experiment, if plants are not studied in the seed germination phase in the presence of the stressor, and for equalizing the conditions, it is pos-

sible to layer the agar medium without NaCl of various heights to the first phase of the salt medium.

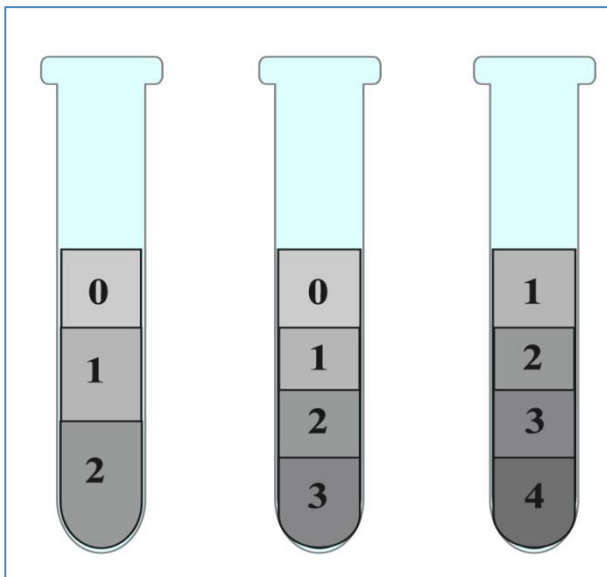


Figure 2. Scheme of the variants with the use of salt stress gradient. Agar medium without stressor. 1;2;3;4-agar media (blocks) with increasing concentration of the stressor factor.

Seed preparation: The process of plant seed preparation is their sterilization for the subsequent placement in the agar medium.

Seed sterilization: Plant seeds were thoroughly washed with running water with the addition of detergent, and kept in a weak solution of KMnO_4 for 10-15 minutes. All other manipulations related to the sterilization process were carried out under aseptic conditions. The first stage of sterilization involved incubating the seeds in a 10-12% solution of H_2O_2 for 10 minutes, washing with sterile water 2-3 volumes from the volume of the sterilizing solution. The second stage consisted of the sterilization with 70% ethyl alcohol for 5 minutes followed by washing with 3 volumes of sterile water. The third and final stage was sterilization with Na hypochlorite with the addition of Tween-20 detergent and careful washing with 5-6 volumes of sterile water. The period of incubation in sterile water between new portions should be at least 5 minutes. The duration of sterilization and concentration of the sterilizing agent must be selected experimentally to avoid both the excess effect of the sterilizer and its insufficient effect. The optimal concentration, when using Na hypochlorite with 5% active substance, the sterilization period was 15-20 minutes. The main condition was the stabilization of the temperature of the sterilizing solution (20-25°C).

Prepared in this way sterile seeds are planted in vessels with agar medium. When placing seeds on the surface of the agar medium, it is desirable to add ~ 1ml of sterile water in order to create the necessary humidity for rapid swelling and seed germination. After 2-3 days, the seeds germinated at a temperature of 23-25°C, in the dark and after germination they were transferred to light with the necessary intensity of illumination, humidity and temperature.

In the initial period of plant growth and development, the regulation of moisture does not matter, since it remains in the vessels to a sufficient degree. When seedlings reached the level of a cotton plug, it was removed, and the plants in the vessels were grown till the agar dried. Usually this period (depending on the volume of the agar block) is 40 to 60 days.

It is possible to maximally prolong the time of the experiment to study the influence of various factors at certain stages of ontogenesis. In this case, as the agar block dries, it is replaced by a neutral substrate. The most acceptable is the use of perlite. In this case, only the degree of wetting of perlite by the nutrient solution is monitored in the same proportions as those used in the preparation of the agar medium. In this case, the approach used is a successive change of agaroponics by agregatoponics.

The advantage of the methodical approach with the use of agar is that the growth processes of the vegetative part of plants and root system can be observed and controlled simultaneously (Fig. 3).

Growth and development of roots depend on the environmental conditions. Survival under heterogeneous soil conditions is determined by the plasticity of the roots to avoid unfavorable factors, such as salinity. Root resistance to salt and other stressors is a component of the overall resistance of the organism, and in some cases, depending on the type of stressor, the plant roots which are the structural and functional part of the body, are the first subjected to stress. The complexity of the root system responses to a stressor was confirmed by transcriptomics and proteomics (Koussevitzky et al, 2008; Rasmussen et al, 2013; Rivero et al, 2014; Sewelam et al, 2014).

The proposed culture system allows perfect controlling and modeling the relationship between the development of the plant vegetative part and the root system, in the presence of single, double and multiple stress, and studying the resistant variability to counteract stress combinations (Fig. 4).



Figure 3. Development of the root system of wheat plants using agaroponics.

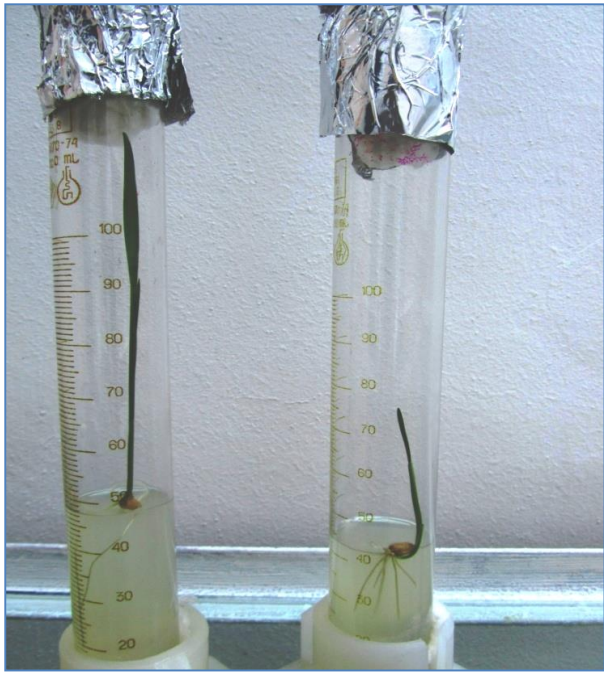


Figure 4. Development of wheat plants in agar medium:
1 - without stress factor; 2- in the presence of 100 mM NaCl.

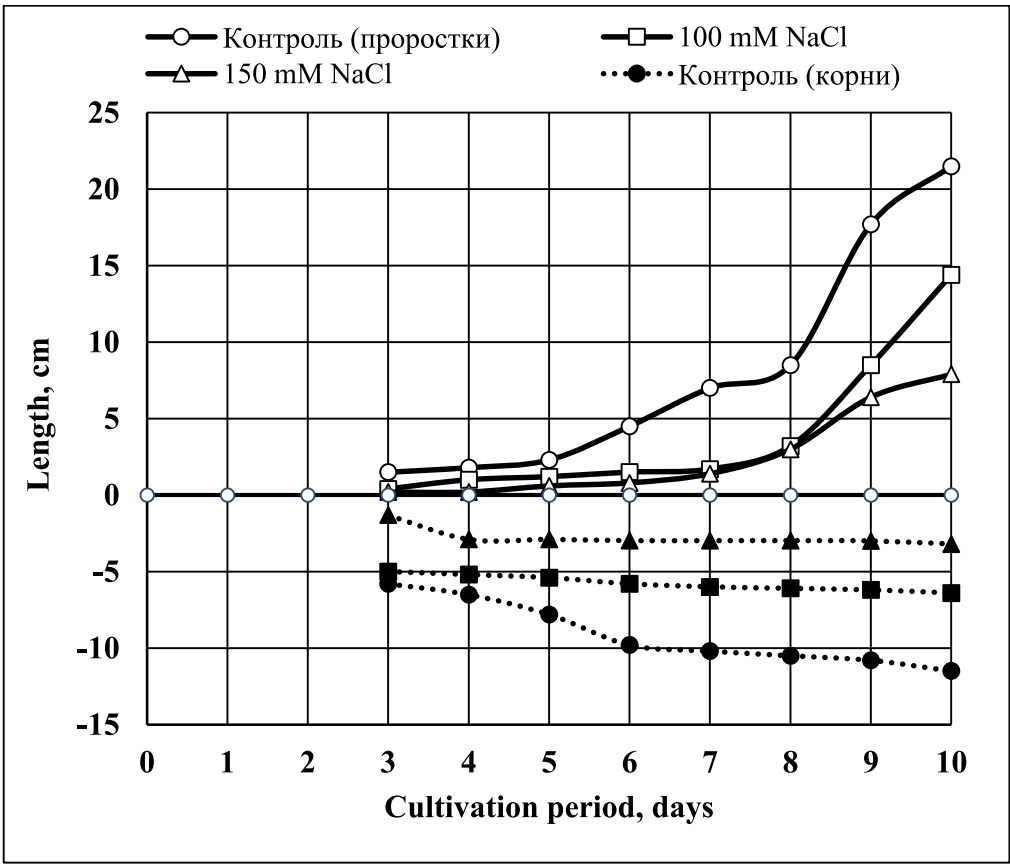


Figure 5. Response of durum wheat plants to salt stressor.

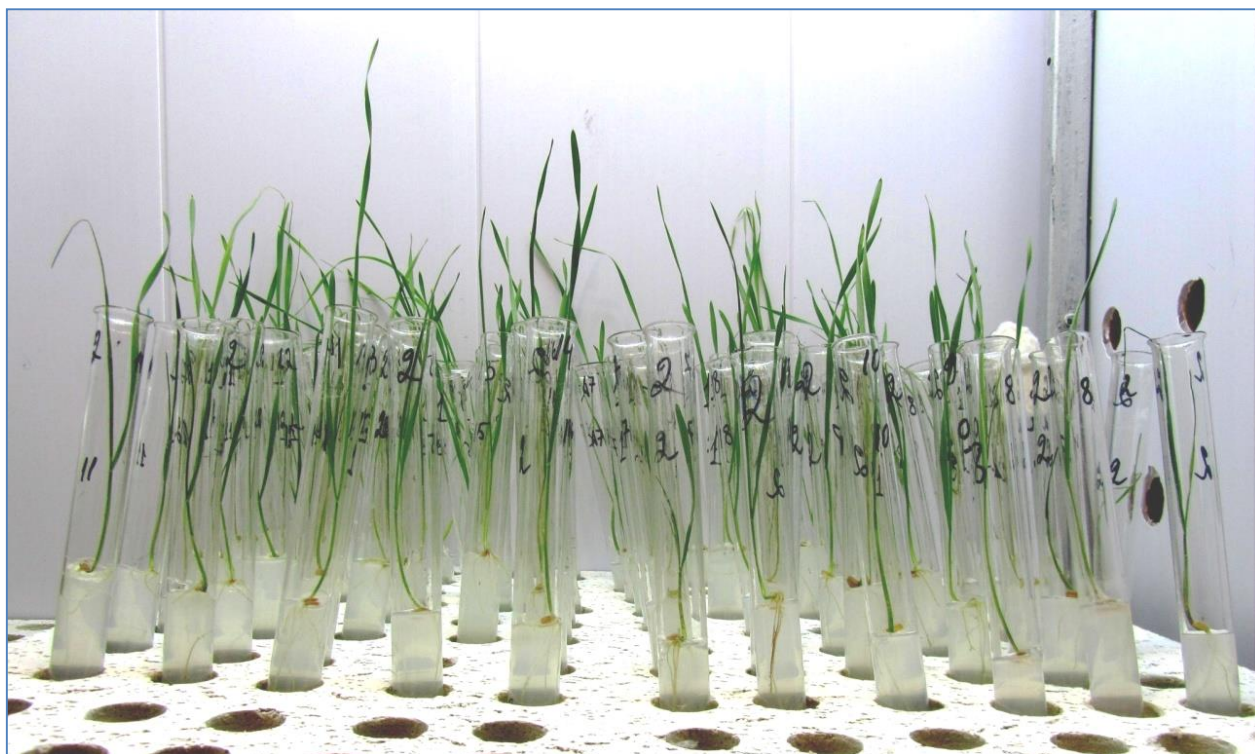


Figure 6. Cultivation of wheat genotypes under different levels of salt stress.

When adapting to constantly changing conditions, plants need to adapt their development program. These processes largely depend on changing conditions. The level of plasticity of the root system can facilitate the response to stress (Pierik et al., 2014). Recently, the studies of root adaptations have become increasingly important.

So, in particular, the root morphology and their functionality can be influenced by a number of factors - mineral supply, osmotic stress, light, salinity (Giehl et al., 2014; Malamy, 2005; Galvan-Ampudia et al., 2011; Kellermeier et al., 2014).

Figure 5 presents (as an example) the results of the study of the dynamics of durum wheat development under salt stress. The results of 10-day cultivation allow us to get an idea of the separate influence of the salt stressor on the growth indices of the vegetative and root system of plants, depending on the level of the stress factor.

This methodological approach makes it possible to establish the limits of resistance to the level of salt stressor, to establish the correlation of the responses of the vegetative and root parts of the plant organism and to determine the response, adaptability and resistance to salt stress.

The described method was used to study the reaction to salt stress of more than 18 genotypes of durum and bread wheat from the genebank of the Institute of Plant Resources of the National Academy of Sciences (Fig. 6).

Based on the obtained data, the genotypes are ranked according to the degree of resistance to salt stress. Thus, this method can be applied in a wide range of studies on the influence of various factors on the growth and adaptive processes of plants. It is particularly valuable in modeling the growing effects, including combined effects on the root system and allows performing researches and observations on the same object.

This experimental approach can also be used to study the pathogen-host interaction both simultaneously in the root and vegetative relationships, or separately, to study the genetic control of the virulence of populations of the causative agent of diseases, in the selection of varieties with horizontal resistance, for the selection of genotypes with host stability in order to increase its variability amplitude (Korobova et al., 2013; Tyryshkin et al, 2000).

Obviously, this method is applicable not only for working with cereals, but also for other plants when studying the action of various stressors. Primary stability diagnostics, combined with studies of adaptive capabilities, as a test is important and necessary for selection processes.

ACKNOWLEDGEMENT

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**Bitki Orqanizminə Biotik Və Abiotik Təsirin Modelləşdirilməsi Üçün
Ağar Sistemi (Agaroponika)
(Metodiki Təvsiyə)**

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Aqaroponikadan istifadə etməklə duz stresinin modelləşdirmə metodu təsvir edilmişdir. Buğda bitkisinin rezistentlik və adaptivliyinin öyrənilməsində stressor təsirin artan qradient üzrə modelləşdirilməsinə yanaşmalar nəzərdən keçirilib

Açar sözlər: *Aqaroponika, stressor, duz stresinin qradienti, modelləşdirilmə, buğda*

**Агаровая Система (Агаропоника) Для Моделирования Абиотических
И Биотических Воздействий На Растительный Организм
(Методическая рекомендация)**

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Предложен метод использования агаропоники для изучения резистентности и адаптивности растений пшеницы к стрессорным воздействиям. Показана возможность моделирования нарастающего солевого стрессорного фактора.

Ключевые слова: *Агаропоника, стрессор, градиент солевого стресса, моделирование, пшеница*

The Comparative Analysis of the Taxonomic Composition of *Boraginaceae* Juss. Distributed in Azerbaijan and Phytogeographic Regions of Adjacent Countries

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This article presents the comparative analysis of genus and species of *Boraginaceae* Juss. distributed in Azerbaijan and its neighboring countries (the Dagestan Autonomous Republic (Russian Federation), Georgia, Armenia, Turkey and the Islamic Republic of Iran). In order to compare the similarity of plant species distributed in two neighbor countries the floristic similarity coefficient was determined using the floristic similarity coefficient of Jaccard index. The results of the analysis showed that *Boraginaceae* distributed in Azerbaijan has a closer commonness with the flora of Armenia, Georgia and Dagestan for its taxonomic composition, than with the floras of Turkey and Iran. This may be attributed to the degree of comparison of the measured areas of the investigated territories, the type of areal or geographic elements that affect the formation of these territories, similarity and differences in climatic conditions.

Keywords: Phyto-geographical regions, *Boraginaceae* Juss., Jaccard method, similarity indices, taxon of the flora of Azerbaijan

INTRODUCTION

In order to come to a final conclusion about the species composition and systematics of *Boraginaceae* Juss. distributed in Azerbaijan it is not enough to study the systematics composition of species belonging to this family and distributed only in the Republic separately. In order to reach our goal, it will be appropriate to carry out a critical study of the representatives of *Boraginaceae* distributed in the floras of neighboring countries of Azerbaijan and make a comparative analysis of their similar and different properties.

The article gives the comparative analysis of species of the family *Boraginaceae* Juss. distributed in Azerbaijan with ones distributed in phytogeographical regions surrounding Azerbaijan. As we know the Republic of Azerbaijan is bordered by the Caspian Sea in the East, the Russian Federation in the North (the Dagestan Autonomous Republic), the Republic of Georgia in the North-West, the Republic of Armenia in the West, the Republic of Turkey in the South-West (the Nakhchivan Autonomous Republic) and the Islamic Republic of Iran in the South (Figure 1). If we glance at the general number of *Boraginaceae* distributed in the flora of the mentioned phyto-geographical regions, then we will notice that this number consists of 473 species belonging to 54 genera which are not so low in comparison with the general area of the studied countries. In fact, if we determine that 502 species belonging to 54 genera are distributed in the study

areas, it is supposed that 29 species are distributed in these areas and as the distribution of these species is not confirmed completely, then we do not consider these species in our studies. We can only briefly note that the number of species of which is probably distributed in the flora of Azerbaijan is 8 species (*Heliotropium dolosum*, *Onosma dichroantha*, *O. setosa*, *O. isauricum*, *Nonea pulla*, *Lycopsis arvensis*, *Myosotis sylvatica*, *Lappula marginata*), in the flora of Turkey – 14 species (mainly *Onosma* – 7 species, *Alkanna* – 1 species, *Anchusa* – 1 species, *Nonea* – 1 species, *Pulmonaria* – 2 species, *Moltkia* – 2 species), in the flora of Iran – 21 species (*Heliotropium* – 4 species, *Arnebia* – 3 species, *Onosma* – 3 species, *Nonea* – 2 species, *Myosotis* – 4 species, *Eritrichium* – 1 species, *Lappula* – 1 species, *Cynoglossum* – 1 species, *Solenanthus* – 1 species, *Paracaryum* – 1 species).

The results of the first studies showed that totally 31 species belonging to 18 genera such as *Argusia sibirica*, *Heliotropium ellipticum*, *H. europaeum*, *H. suaveolens*, *Huynhia pulchra*, *Lithospermum officinale*, *Aegonichon purpureo-coeruleum*, *Buglossoides arvensis*, *B. tenuiflora*, *Onosma sericeum*, *O. microcarpa*, *Cerinthe minor*, *Echium maculatum* (*E. russicum*), *E. italicum*, *Nonea lutea*, *N. rosea*, *N. caspica*, *Symphytum asperum*, *Anchusa italica*, *Lycopsis orientalis*, (*Anchusa arvensis*, subsp. *orientalis*), *Myosotis micrantha* (*M. stricta*), *M. arvensis*, *M. caespitosa*, *M. lithospermifolia*, *Strophostoma sparsiflora* (*M. sparsiflora*), *Asperugo procumbens*, *Lappula*

barbata, *L.squarrosa*, *L.patula*, *Cynoglossum officinale* and *C.creticum* are common species encountered in the flora of all studied countries. This comprises about 6% of general number of species distributed in the region. If we glance at the number of common species we will notice a different picture. 21 genera out of 54 of which species distributed in the studied geography are represented in the flora of Azerbaijan and its surrounding countries. This number comprises about 39% of common species distributed in the studied geography. These percentages can also be accepted as the similarity coefficient of the family *Boraginaceae* for the studied general geography.

MATERIALS AND METHODS

Studies were carried out in nature and herbarium materials of the family *Boraginaceae* in 2000-2016 (Karimov, 1999, 2000, 2013, 2014, 2016 a, b, c). Materials kept at the Institute of Botany of Russian Academy of Sciences (RAS), the Herbarium Foundation in Tbilisi and the Herbarium Foundation of the Institute of Botany of ANAS were ana-

lyzed. References were made to the literature and internet resources, various maps and the results of monitoring's carried out by authors in nature (Flora of Armenia, 1980; Flora of Georgia, 1985; Flora of Caucasus, 1967; Flora of Azerbaijan, 1957; Flora of Turkey, 1978; Flora of Iran, 2002; Grossheim 1936, 1948; Cherepanov, 1995; Asgarov, 2011; Khokhryakov, 1993; Murtuzaliyev, 2009). Comparative morphological, systematical, botanical, florogenetic and other methods were used during the study.

The article presents the comparative analysis of the taxonomic composition of *Boraginaceae* of 5 phyto-geographical regions neighboring with Azerbaijan on the basis of the floristic similarity coefficient of Jaccard. As a result similarity coefficient of genus and species was determined.

RESULTS AND DISCUSSION

In order to form a clear picture about *Boraginaceae* of the widely studied geography it is important to review the modern condition of some taxa belonging to this family.

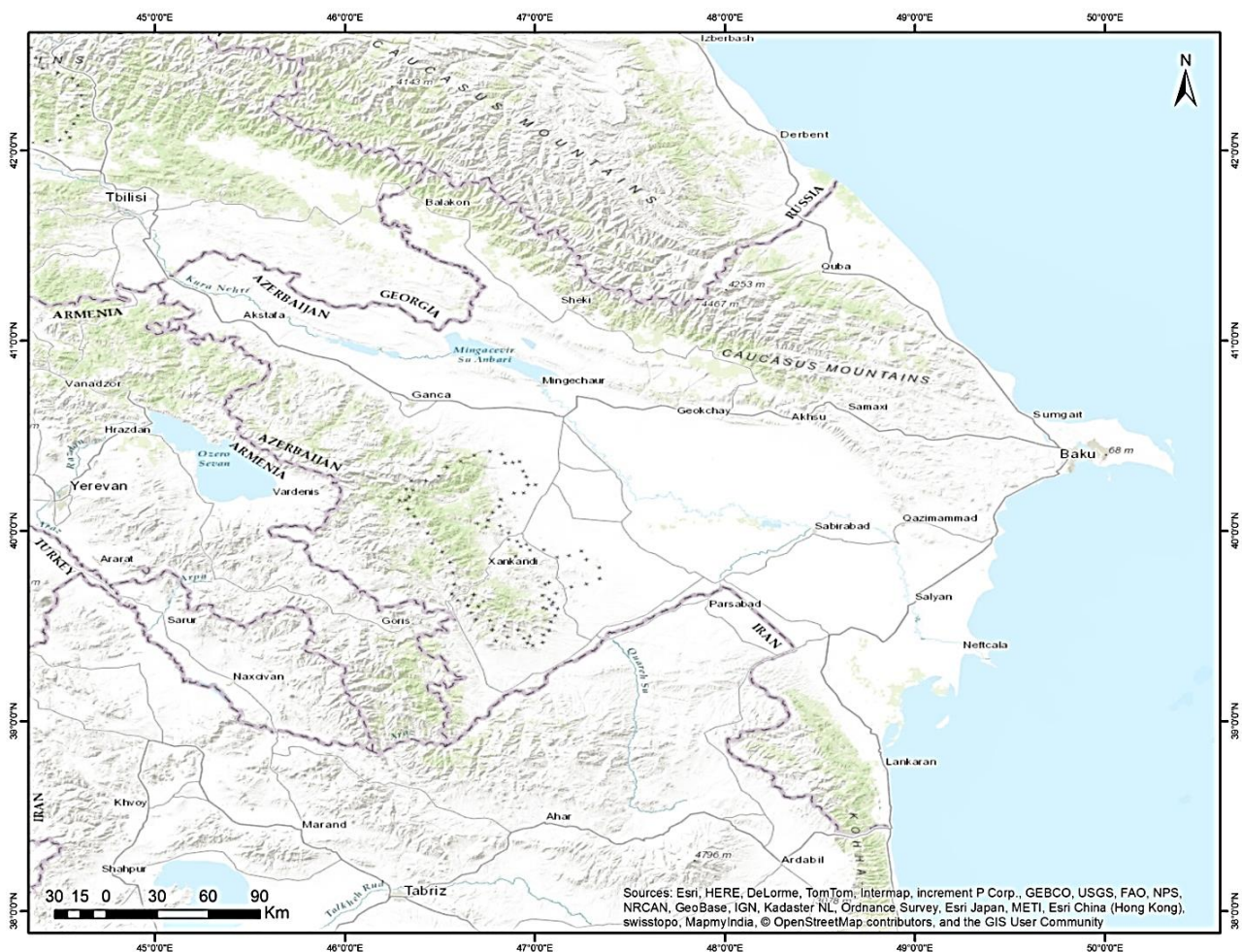


Figure 1. Map of Azerbaijan Republic and surrounding phytogeographical regions.

Two genera - *Hormuzakia* Gusuleac and *Phyllocara* Gusuleac, which were described by Gushulyakh are separated from the genus *Anchusa* in the flora of Iran (Flora Iranica, 2002), where the same genera are recorded as a subgenus - subgen. *Hormuzakia* (Gusuleac) Chamb. and subgen. *Phyllocara* (Gusuleac) Chamb. in the flora of Turkey. As both of these descriptions are justified, but taking into consideration that Gushulyakh was described before and is high-rank taxon in terms of systematics, we accept both genera in the genus status for the flora of both Turkey and Iran and carry out our comparison. On the other hand, Buassy separated several species belonging to the genera *Paracaryum* and described separately as the genus *Mattiastrum* Boiss. This taxon is presented as subgen. *Mattiastrum* (Boiss.) R.Mill. in semi-genus status in the flora of Turkey and Iran. This taxon is given in an independent genus status in several modern literature sources (Murtuzaliyev, 2012). We accept this approach to be true and present the species referred to this genus by Buassy which are distributed both in Azerbaijan and its neighbouring countries in the composition of the genus *Mattiastrum* (Boiss.) R.Mill. A species *P.imeretinum* M.Pop. (*P.glochidiatum* (Wall. ex Benth.) Popov ex Czukav) which is belonging to other genus *Paracynoglossum* M.Pop. described by M.G.Popov from Georgia, were also determined in the flora of Turkey. But the difference is that here this species is presented as species *Cynoglossum glochidiatum* Wall. ex Benth. If we consider that this species is described for morphological properties differed markedly from other species of genus *Cynoglossum*, mainly for properties with constant and diagnostical importance in the structure of flower and fruit, then we accept *Paracynoglossum* in the genus status and carry out our comparative analysis on this basis. Besides the matter that tens of species in these floras are named on the basis of personal approaches of separate authors is clarified by

taking as a basis the revisions of S.K.Cherepanov (1995). Without applying these revisions it will be impossible to clarify the taxonomic composition of *Boraginaceae* of region as a whole, as well as separate phyto-geographical regions.

The family *Boraginaceae* is presented with 107 species belonging to 32 genera in the flora of the Republic of Azerbaijan. 73 species belonging to 26 genera of this family are found in the Dagestan Autonomous Republic of Russian Federation, north neighbor of Azerbaijan, 92 species belonging to 35 genera of this family in the Republic of Georgia, north-west neighbor of Azerbaijan, 83 species belonging to 29 genera of this family in the Republic of Armenia, west neighbour of Azerbaijan, 302 species belonging to 41 genera of this family in the Republic of Turkey, south-west neighbor of Azerbaijan and 218 species belonging to 46 genera of this family in the Islamic Republic of Iran, south neighbor of Azerbaijan (Figure 2).

The comparative analysis of the family *Boraginaceae* of Azerbaijan and its surrounding regions was carried out, similarity coefficient on genus and species were determined and the following conclusions are provided (Table 1):

1. As mentioned above the family *Boraginaceae* is represented with 73 species belonging to 26 genera in the flora of the Dagestan Autonomous Republic (Russian Federation). Species belonging to 8 genera such as *Caccinia*, *Suchtelenia*, *Alkanna*, *Paracarium*, *Heterocarum*, *Rindera*, *Arnebia* and *Moltkia* included to the list of Azerbaijan *Boraginaceae* are not found in the Dagestan Autonomous Republic and contrary species belonging to 2 genera such as *Trigonotis* and *Pulmonaria* encountered in the flora of Dagestan are not found in the flora of Azerbaijan. So the number of common species found both in the flora of Azerbaijan and Dagestan is 24.

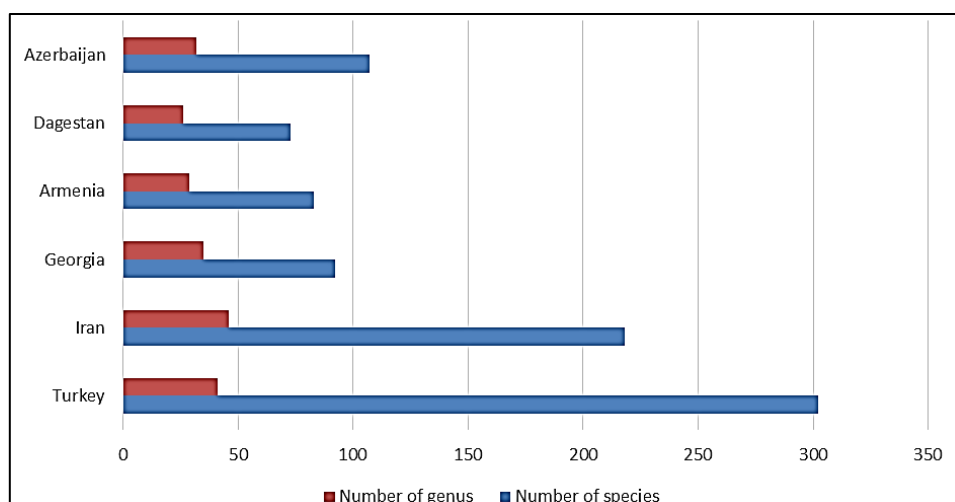


Figure 2. Number of genera and species of *Boraginaceae* of Azerbaijan and surrounding phyto-geographical regions.

Table 1. *Boraginaceae* Juss. species occurrences and their comparative analysis at the Azerbaijan and its surrounding phytogeographical regions

[illegible]

A – The number of common species of genus distributed in both Azerbaijan and in the noted country;

B – The number of species of genus which is distributed in Azerbaijan, not distributed in the noted country;

C – The number of species of genus which is distributed in the noted country, not distributed in Azerbaijan.

In order to determine the number of common species of *Boraginaceae* distributed in both regions and how they close to each other in terms of taxonomic composition, the above mentioned the floristic similarity coefficient of Jaccard was used (Jaccard, 1912; Sukhachev, 1930):

$$k = \frac{a \cdot 100}{b}$$

here K is the floristic similarity coefficient, a—the number of common species or genera of the compared areas, b—general number of species and genera found in these areas. So if we consider that general number of species of genera of the family *Boraginaceae* found in the floras of Dagestan and Azerbaijan is 34, the number of common species is 24, then the floristic similarity coefficient will be as $k=(24 \times 100\%):(32+2)=\sim 71\%$ according to the calculation by Jaccard similarity index.

The picture of the comparison of species will be a little bit different. So while 63 species of *Boraginaceae* out of 107 found in the flora of Azerbaijan are encountered in the Dagestan Autonomous Republic, only 10 species out of 73 distributed in Dagestan are not encountered in Azerbaijan (Murtuzaliyev, 2009).

So the general number of species of the family *Boraginaceae* found in the flora of Dagestan and Azerbaijan is 117, the number of common species-63. If we estimate the floristic similarity coefficient, we will obtain $k=(63 \times 100\%):117=\sim 54\%$. As the floristic similarity coefficient of species is assumed as a basis, we can surely note that the floristic similarity coefficient of *Boraginaceae* of these two neighbor regions is $\sim 54\%$. Though these regions are adjacent and have almost the same climate condition, the difference can be probably explained with the existence of impassable mountains always covered in snow which separates these regions from each other.

2. Species belonging only to 5 genera -*Borago*, *Pulmonaria*, *Trachistemon*, *Paracynoglossum* and *Eritrichium* out of 92 species belonging to 35 genera of *Boraginaceae* distributed in the Republic of Georgia are not found in Azerbaijan. In defiance only two genera -*Rindera* and *Paracaryum* are not represented in the flora of Georgia, it became clear from the first view that the floristic composition of these two regions are close (Flora of Georgia, 1985). So if we consider that the general number of genera is 37, the number of common genera is 30, then we will observe that the floristic similarity coefficient of genera belonging to the family *Boraginaceae* of these regions is $k=(30 \times 100\%):37=\sim 81\%$.

But the comparison of species is completely different. The number of species of *Boraginaceae*

encountered in Georgia out of 107 species distributed in the flora of Azerbaijan is 67. Though 25 species are found in the flora of Georgia, we can note that these species are not encountered in Azerbaijan. The general number of floristic species of these regions is $107+25=132$, the number of common species is 67. If we calculate the floristic similarity coefficient we will notice it to be as $k=(67 \times 100\%):132=\sim 51\%$. In comparison with the similarity percentages of genera the reason why similarity percentages of species is so low can be explained with a big difference in the number of species in the genera as *Omphalodes* and *Nonea*. While genus *Omphalodes* is represented with one species in Azerbaijan, 7 species of this genus are found in Georgia or while genus *Nonea* is represented with 18 species in Azerbaijan, distribution of totally 9 species of this genus in the flora of Georgia is shown which stipulates the similarity coefficient of species to be relatively small number. On the other hand, it is explained with the fact that the elements of Europe and Caucasus have a relative great influence on the formation of the flora of Georgia in comparison with the flora of Azerbaijan (Flora of Georgia, 1985; V.Z. Gulisashvili, 1964). It shall be noted that flora elements of Mediterranean Sea have a great influence on the flora of Azerbaijan.

3.If we compare the *Boraginaceae* of the flora of Armenia, the additions of K.G.Tamanyan who prepared this family for publication of new flora shall be taken into consideration. According to the analysis we can note that the family *Boraginaceae* was represented with 83 species belonging to 29 genera in Armenia (Flora of Armenia, 1980; Tamanyan K.G., 2011). Species belonging to 4 genera such as *Brunnera*, *Omphalodes*, *Suchtelenia* and *Trigonocaryum* distributed in the flora of Azerbaijan are not totally found in the flora of Armenia. There is visual evidence that besides the geographic features of Caucasus, the features of ancient Mediterranean Sea have an influence on the formation of the flora of Armenia.

Species *P.molissima* belonging to genus *Pulmonaria* L. of which only one genus is found in the flora of Armenia is represented in the flora of Azerbaijan. This is also an example of rich flora of Azerbaijan in comparison with the flora of west neighbor. So if we consider that the general number of *Boraginaceae* distributed in the flora of Azerbaijan and Armenia is $32+1=33$, the number of common genera is 28, so the similarity coefficient of genera of these neighbour countries will be as $k=(28 \times 100\%):33=\sim 85\%$.

So if we consider that the general number of common species of *Boraginaceae* distributed in the flora of these regions is 72 and only 11 species of *Boraginaceae* distributed in the flora of Armenia

are not found in the flora of Azerbaijan, then the similarity coefficient for these both regions will be as $k=(72 \times 100\%):118 \approx 61\%$.

4. If we pay attention to the taxonomic composition of *Boraginaceae* distributed in the flora of Turkey, south-west neighbour of Azerbaijan we will observe that this family is represented with 302 species belonging to 41 genera out of which species belonging to 11 genera such as *Cordia* L., *Trachystemon* D. Don, *Pulmonaria* L., *Lithodora* Griseb., *Trachelanthus* Kunze, *Trichodesma* R. Br., *Borago* L., *Paracynoglossum* M.Pop., *Neatostema* Johnston, *Hormuzakia* Gusulaec and *Phyllocara* Gusulaec are not found in the flora of Azerbaijan. The species of genera such as *Trigonocaryum* and *Suchtelenia* found in the flora of Azerbaijan are not distributed in the flora of Turkey. So if we take into consideration that the number of common genera is 30, the total number of genera is 43, then the similarity coefficient of genera for both regions will be $k=(30 \times 100\%):43 \approx 70\%$.

229 species distributed in the flora of Turkey are not found in the flora of Azerbaijan, contrary 34 species distributed in the flora of Azerbaijan are not found in the flora of Turkey. If we consider that the number of common species in both regions is 73, the general number of species is $107+229=336$, then the similarity coefficient of species will be as $k=(73 \times 100\%):336 \approx 22\%$. The reason why the similarity coefficient of species is so different and low can be explained in the following way: several genera represented with a lower number in Azerbaijan are distributed with numerous species in Turkey. For example: the genus *Onosma* is represented with 10 species in Azerbaijan, 88 species in Turkey, the genus *Alkanna* is represented with only 1 species in Azerbaijan, 31 species in Turkey, the genera *Symphutum* and *Anchusa* are represented with 3 species in Azerbaijan, 20 and 14 species in Turkey which stipulates the difference and low percentage of the similarity coefficient of species.

5. The flora of Iran is represented with many Taxons- 218 species belonging to 46 genera as in the flora of Turkey, which is explained as follows: the area of Iran is nearly 10 times larger than the area of other regions and the flora is formed under the influence of geographical elements of the ancient Mediterranean Sea, Indian Himalaya, Sahara, Irani-Turan and etc.

Species belonging to 15 genera out of 46 such as *Cordia* L., *Ehretia* L., *Tournefortia* (L.) Dandy., *Echiochilon* Desf., *Moltkiopsis* I. M. Johnst., *Gastrocotyle* (Bge.) Benth. et Hooker, *Phyllocara* Gusulaec, *Hormuzakia* Gusulaec, *Eritrichium* Schrad., *Lepechiniella* M.Pop., *Trichodesma* R.Br., *Microparacaryum* (Pop.) Hilger et Podlech, *Helio-carya* Bunge, *Trachelanthus* Kunze and *Lindelofia*

Lehm. encountered in the flora of Iran are not found in the flora of Azerbaijan. Similarly only one genus –*Trigonocaryum* distributed in the flora of Azerbaijan is not represented in the flora of Iran. So if we take into consideration that the number of common genera represented in Iran and Azerbaijan is 31, the general number of genera is 47, then the similarity coefficient of common genera for these countries will be as $k=(31 \times 100\%):47 \approx 66\%$.

If we pay attention to species then we will observe that 45 species distributed in Azerbaijan are not found in the flora of Iran and at the same time 156 species distributed in Iran are not found in the flora of Azerbaijan. As the general number of species of these neighbour regions is $218+45=263$, the number of common species is 62, then the similarity coefficient of species will be $k=(62 \times 100\%):263 \approx 23,5\%$.

If we compare *Boraginaceae* of Iran and Azerbaijan, the reason why the similarity coefficient of species as in the comparison with the flora of Turkey are so low, can be explained in the following way: the genera such as *Onosma* (37 species), *Heliotropium* (31 species), *Paracaryum* (19 species) and *Rindera* (6 species) are represented with numerous species in the flora of Azerbaijan and *Trichodesma* of which none species is noted in Azerbaijan is represented with 8 species in the flora of Iran.

The floristic similarity coefficient of species composition of *Boraginaceae* distributed in Azerbaijan is different from one distributed in Iran and Turkey, therefore several questions are arisen: What will show the comparison of flora of Iran and Turkey? How close or different is the influence of geographical and ecological factors for both these countries?

Therefore, we also decided to determine the floristic similarity coefficient for the taxonomic composition of *Boraginaceae* distributed in Iran and Turkey. As mentioned above *Boraginaceae* is represented with 302 species belonging to 41 genera in the flora of Turkey, with 218 species belonging to 46 genera in the flora of Iran. If we pay attention to the genera we can observe that 11 genera represented in the flora of Iran such as *Ehretia* L., *Tournefortia* (L.) Dandy., *Echiochilon* Desf., *Moltkiopsis* I. M. Johnst., *Gastrocotyle* (Bge.) Benth. et Hooker, *Eritrichium* Schrad., *Lepechiniella* M.Pop., *Suchtelenia* Kar.ex Meissn., *Microparacaryum* (Pop.) Hilger et Podlech, *Helio-carya* Bunge and *Lindelofia* Lehm. are not found in the flora of Turkey and contrary five genera represented in the flora of Turkey such as *Paracynoglossum* M.Pop., *Neatostema* Johnston, *Borago* L., *Trachystemon* D. Don, *Lithodora* Griseb. are not found in the flora of Iran. The species of 2 genera aren't recorded

both in flora of Turkey and Iran. If we consider that the general number of genera is 52, the number of common genera is 35, then the floristic similarity coefficient for genera of *Boraginaceae* of these countries will be accepted as $k=(35 \times 100\%):52=67\%$.

While estimating the floristic similarity coefficient for genera of *Boraginaceae* of these countries, it became clear that the number of general species is 433, the number of common species is 96 ($k=(96 \times 100\%):433 \approx 22\%$). Though the similarity coefficient of species is relatively high, the reason of low percentage of species in the comparison of species is that the formation of species proceeds in different direction under the influence of different factors which results in high percentage of endemism in both floras.

The following table shows the comparative analysis, similarity coefficient of genus and species of taxons in the family of *Boraginaceae* of Azerbaijan with its surrounding 5 phytogeographical regions, as well as between *Boraginaceae* of the floras of Iran and Turkey (Table 2).

As shown from the table *Boraginaceae* distributed in Azerbaijan is closer to *Boraginaceae* distributed in Armenia, Dagestan and Georgia for its taxonomic composition (both for genus and similarity coefficient of species), different from *Boraginaceae* distributed in Iran for the similarity coefficient of genera (totally 66%) and *Boraginaceae* distributed in Turkey for the similarity coefficient

of species (totally -22%). This difference is explained by the fact that the area of Iran and Turkey is larger than the area of the Republic of Azerbaijan and the flora of these countries are formed under the influence of more different phyto-geographical properties.

The similarity analyses comparing the number of genera and species of *Boraginaceae* composition per country or 6 phyto-geographical regions surrounding of the Azerbaijan show that only Caucasian countries for number of genera and species have similarity indices above 70%, and 50%, respectively (Table 3).

According to the flora references, when comparing the number of genera and species of *Boraginaceae* present in the Azerbaijan and the other neighbouring countries, the similarity indices (Figure 3) indicate that none of the pairs of countries or regions have a similarity above 85% and 61%, respectively.

The reason why the floristic similarity coefficient of species of *Boraginaceae* found in the flora of Azerbaijan is high in comparison with the Republic of Georgia, Dagestan and Armenia can be explained with the formation of the Caucasus, including Azerbaijan *Boraginaceae* under the influence of Alban flora. A.A.Grossem who analysed the flora of the Caucasus noted a great role of Alban elements in the formation of general Caucasus flora (Grosheim, 1936).

Table 2. Comparative analysis, similarity coefficient of genus and species of taxons in the family of *Boraginaceae* Juss. of Azerbaijan

Phyto-geographical regions surrounding Azerbaijan	Total number of genus (piece)	The number of common genera (piece)	Similarity coefficient of genus (by %)	Total number of species (piece)	The number of common species (piece)	Similarity coefficient of species (by %)
Dagestan (AR)	34	24	71%	117	63	54%
Georgia	37	30	81%	132	67	51%
Armenia	33	28	85%	118	72	61%
Turkey	43	30	70%	336	73	22%
Iran	47	31	66%	263	62	23,5%
Comparison of <i>Boraginaceae</i> between Iran and Turkey						
Turkey	52	35	67%	433	96	22%

Table 3. Similarity indices comparing the *Boraginaceae* present in the phyto-geographical regions surrounding of the Azerbaijan. Jaccard index values for number of genera in the upper triangle. Jaccard index values for number of species in the lower triangle. The highest similarity values are shown in bold.

	Azerbaijan	Dagestan AR	Georgia	Armenia	Turkey	Iran
Azerbaijan	100	71	81	85	70	66
Dagestan AR (Russia)	54	100	69	67	56	47
Georgia	51	51	100	73	73	59
Armenia	61	49	45	100	71	60
Turkey	22	16	21	21	100	67
Iran	23.5	15	16	23	21	100

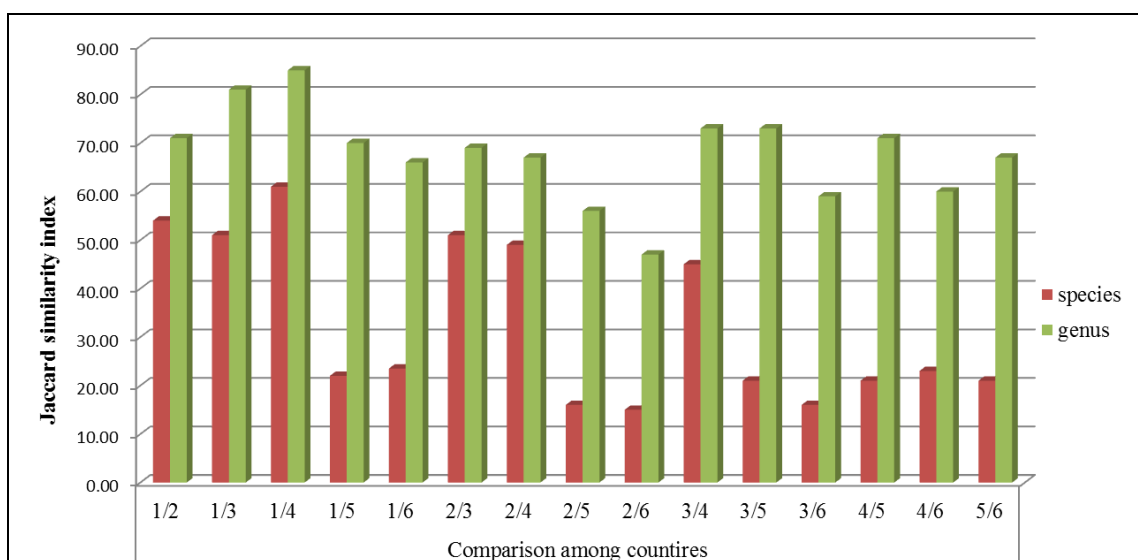


Figure 3. Jaccard similarity index for studied phyto-geographical regions or countries surrounding of the Azerbaijan (1 - Azerbaijan, 2 - Dagestan AR (Russia), 3 - Georgia, 4 - Armenia, 5 - Turkey, 6 - Iran).

On the other, hand species of Azerbaijan *Boraginaceae* have a floristic similarity coefficient with a low percentage in comparison with the flora of the Iran and the Turkey (23,5% and 22% correspondingly), which corresponds to our previous floragenetics outcomes (V.N.Karimov, 2016 a, b, c). It means that while more than 50% (54 species) of 107 species of *Boraginaceae* distributed in Azerbaijan belong to areal types of Caucasus, Atropatan and Europe, a greater part of the flora of Iran and Turkey (70-80%), especially where flora elements of Iran-Turan and Iran-Central Asia played an important role, belong to the areal type of the ancient Mediterranean Sea.

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**Azərbaycan və Onu Əhatə Edən Fito-Coğrafi Regionların Göyzəbankimilərinin
(*Boraginaceae* Juss.) Taksonomik Tərkibinin Müqayisəli Analizi**

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Məqalədə Göyzəbankimilərin (*Boraginaceae* Juss.) Azərbaycan və ona qonşu olan ərazilərdə (Dağıstan Muxtar Respublikası (Rusiya Federasiyası), Gürcüstan, Ermənistan, Türkiyə və İran İslam Respublikası) yayılmış cins və növlərinin müqayisəli analizi verilmişdir. İki qonşu ərazidə yayılmış olan bitki növlərinin oxşarlığını müqayisə etmək üçün Jakarın floristik eynilik əmsalı üsulundan istifadə edərək floristik eynilik əmsalları müəyyənləşdirilmişdir. Aparılan analizin nəticəsi göstərmişdir ki, Azərbaycan Göyzəbankimiləri öz taksonomik tərkibinə görə Ermənistan, Gürcüstan və Dağıstan floraları ilə daha çox yaxın, Türkiyə və İran floraları ilə daha uzaq ortaqlığa malikdir. Bu da öyrənilən ərazilərin sahə ölçülərinin müqayisə olunma dərəcəsi, həmin ərazilərin floralarının hansı areal tipi yaxud da coğrafi elementlərin təsiri ilə formalaşması, nə qədər oxşar və fərqli iqlim şəraitinə malik olmaları ilə izah oluna bilər.

Açar sözlər: Fito-coğrafi regionlar, *Boraginaceae* Juss., Jakar metodu, eynilik əmsalı, Azərbaycan florası taksonları

**Сравнительный Анализ Таксономического Составы Семейства *Boraginaceae* Juss.,
Распространенного В Азербайджане и Фитогеографических Регионах Сопредельных Стран**

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В статье представлен сравнительный анализ распространенных в Азербайджане и соседних с ним странах (Дагестанской Автономной Республике, Российской Федерации, Грузии, Армении, Турции и Иранской Исламской Республике) родов и видов семейства Бурачниковые (*Boraginaceae* Juss.). Для сравнения схожести видов растений, распространенных на двух соседних территориях был использован метод флористической общности - коэффициент Джакара. Результаты проведенных исследований показывают, что Бурачниковые Азербайджана по таксономическому составу более близки к флорам Армении, Грузии и Дагестана, тогда как с флорами Турции и Ирана свойственно более далекое сходство. Это можно объяснить степенью сравнения измеренных площадей исследованных территорий, типом ареала или географических элементов, влияющих на формирование этих территорий, сходством и различием климатических условий.

Ключевые слова: Фитогеографические регионы, *Boraginaceae* Juss., метод Джакара, коэффициент сходства, таксоны Азербайджанской флоры

Study of Biologically Active Compounds of the Roots of *Prangos biebersteinii* Karjag.

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The roots of *Prangos biebersteinii* Karjag. collected from Beshbarmak mountains were investigated. Seven crystalline substances of the coumarin nature were isolated from an acetone extract of *P. biebersteinii* roots using the method of column chromatography (Al_2O_3 , III-IV degree activity): $\text{C}_{16}\text{H}_{14}\text{O}_4$, m.p. 108.0-109.0°C (1), $\text{C}_{15}\text{H}_{16}\text{O}_3$, m.p. 84.0-85.0°C (2), $\text{C}_{16}\text{H}_{14}\text{O}_5$, m.p. 145.0-146.0°C (3), $\text{C}_{16}\text{H}_{16}\text{O}_5$, m.p. 109.0-110.0°C (4), $\text{C}_{16}\text{H}_{14}\text{O}_5$, m.p. 141.0-143.0°C (5), $\text{C}_{16}\text{H}_{16}\text{O}_6$, m.p. 137.0-138.5°C (6), $\text{C}_{11}\text{H}_6\text{O}_4$, m.p. 286.0-279.5°C (7). On the basis of physical and chemical properties (elemental composition, melting temperature) and spectral (UR- and NMR-spectra) data they were identified as isoimperatorin (1), ostol (2), isooxypeucedanin (3), pranferol (4), peucedanin (5), oxypeucedanin hydrate (6) and bergaptol, respective (7).

Keywords: *Prangos*, roots, sum of substances, chromatography, individual, UR-spectroscopy, NMR-spectroscopy, chemical shift.

INTRODUCTION

The species which on morphological features quite differs from *Prangos ferulacea* species I.I.Karjagin in agreement with A.A.Grossheim named as *Prangos biebersteinii* in honor of M.Bieberstein who collected and separated it from the East Caucasian "race" for the first time (Karjagin, 1955). But recently, the species *P. biebersteinii* was merged with *Prangos ferulacea* (L.) Lindl. and represent as a synonym of the latter species (Menitsky, 2008).

Literature data on chemical studying of *P. biebersteinii* is relatively rare (Abyshev et al., 1973, 2003; Abyshev, Brodsky, 1974).

The studied plant species *P. biebersteinii* is characterized by the presence in their composition of biologically active coumarin derivatives as well as most representatives of *Apiaceae* family. Conversely there are a great number of scientific works devoted to the chemical study of *P. ferulacea* which is merged to this species (Abyshev, 1969, 1974; Abyshev et al., 1972, 1973, 1974; Kuznetsova, Abyshev, 1965a, 1965b).

MATERIAL AND METHODS

The research object representing the dried and finely ground roots of *Prangos biebersteinii* Karjag. (155 g) which were collected in 09.05.2014 from Beshbarmag mountains in the flowering phase was extracted with acetone. Output of the sum of extractive substances was 5.16%. For isolation of individual compounds the 8.0 g extractive substances subjected to the column chromatography method in

columns ($h=45$ cm, $d=2.5$ cm) filled with neutral (with III-IV activity degree) Al_2O_3 . An identity of obtained substances using of thin layer chromatography on Silufol UV-254 plates is confirmed. An individual compounds based on physical-chemical (elemental composition, melting point) properties and on information received at the detection of IR- and NMR-spectra were identified. IR-spectra in Varian 640-IR spectrometer, NMR-spectra in Bruker 300 spectrometer at the 300 MHz resonance frequency in DMSO-d_6 solvent were registered. The melting points (m.p.) of individual compounds in Boethius table were determined.

RESULTS AND DISCUSSION

From the fractions obtained as a result of elution of chromatographic column with solvents hexane, benzol, chloroform and their mixtures in different ratios from the sum of extractive substances of *Prangos biebersteinii* Karjag. roots 7 compounds in individual state have been isolated.

Compound-1. From fractions of 1-2 eluted with hexane a compound with elemental composition of $\text{C}_{16}\text{H}_{14}\text{O}_4$ and melting point (m.p.) of 108.0-109.0°C was obtained.

IR-spectrum of compound contains absorption bands relating to carbonyl group of δ -lactone ring (1723 cm^{-1}) and double bonds of aromatic system ($1626, 1601, 1579, 1544\text{ cm}^{-1}$). The studied compound 1 has been identified as izoimperatorin by comparison of its IR-spectra with IR-spectra of known coumarin derivative isoimperatorin (Serkerov and Aleskerova, 2006).

Compound-2. The elemental composition and melting point of the compound 2 obtained from fractions of 5-6 eluted by hexane were $C_{15}H_{16}O_3$ and m.p. of 84.0-85.0°C, respectively.

In the area of characteristic IR-spectrum absorption frequencies the bands of lactone ring carbonyl group (1721 cm^{-1}) and aromatic system double bands ($1604, 1564, 1498\text{ cm}^{-1}$) have been revealed. The signals revealed in ^1H NMR-spectrum of compound: two singlets with 3H area of each (1.60; 1.80 ppm), doublet with 2H area (3.40 ppm, $J=4.5\text{ Hz}$) and triplet with 1H area (5.11 ppm, $J=4.5\text{ Hz}$) prove the existence of open side chain ($-\text{CH}_2-\text{CH}=\text{C}(\text{CH}_3)_2$) consisting 5 carbon atoms in molecule. The signals detected in lower magnetic field of spectrum: 6.20 (d., $J=9.65\text{ Hz}$, 1H, H-3), 7.91 (d., $J=9.65\text{ Hz}$, 1H, H-4), 7.50 (d., $J=9.10\text{ Hz}$, 1H, H-5) and 7.00 ppm (d., $J=9.10\text{ Hz}$, 1H, H-6) characterize double bonds of aromatic cycle of the compound. The singlet with chemical shift of 3.89 ppm in spectra is the evidence of methoxy-group ($-\text{OCH}_3$) in the structure of the studied compound.

Thus, the results obtained from the detection of IR- and ^1H NMR-spectra indicate that the structure of the compound 2 is identical with ostol (Gasimova, Serkerov, 2011).

Compound-3. The elemental composition and melting point of the compound obtained from fractions eluted by mixture of benzole and chloroform (2:1) were $C_{14}H_{14}O_5$ and of 145.0-146.0°C, respectively. In IR-spectrum of the compound absorption bands relating to δ -lactone cycle (1744 cm^{-1}), ketone group (1616 cm^{-1}) and double bonds of aromatic system ($1622, 1579, 1546, 1513\text{ cm}^{-1}$) have been revealed. By direct comparison of IR-spectrum of studied compound with IR-spectrum of isooxypeucedanin compound 3 was identified as isooxypeucedanin (Serkerov, Aleskerova, 2006).

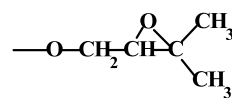
Compound-4. The elemental composition and melting point of the compound obtained from fractions of 78-80 of the chromatographic column eluted by mixture of benzole and chloroform (1:1) were $C_{16}H_{16}O_5$, m.p. 109.0-110.0°C, respectively. In the area of characteristic IR-spectrum absorption frequencies the bands characterizing of hydroxyl group ($3450\text{--}3200\text{ cm}^{-1}$), $\text{C}=\text{O}$ group of δ -lactone cycle (1706 cm^{-1}) and double bonds of coumarin structure ($1628, 1619, 1575, 1547\text{ cm}^{-1}$) were detected.

In IR-spectrum of the studied compound there are two intensively bands in the area of $1600\text{--}1650\text{ cm}^{-1}$. This is characteristic for spectra of 5-mono-substituted furocoumarins, for example oxypeucedanin. However, for spectra of 8-mono-substituted furocoumarins, for example in the same area of spectra of the prangenin, prangenin hydrate, imperatorin only one weak band ($1625\text{--}1620\text{ cm}^{-1}$) appears (Kuznetsova, 1967).

Thus taking into account abovementioned facts and also directly comparing IR-spectra of pranferol and studied coumarin derivative the compound 3 was identified with 5-mono-substituted furocoumarin - pranferol (Serkerov, Aleskerova, 2006).

Compound-5. The elemental composition and melting point of the compound obtained from fractions of 88-91 of the chromatographic column eluted by mixture of benzole and chloroform (1:2) were $C_{16}H_{16}O_5$, m.p. 141.0-143.0°C correspondingly. In the area of characteristic IR-spectrum absorption frequencies the absorption bands relating to $\text{C}=\text{O}$ group of δ -lactone cycle (1735 cm^{-1}) and double bonds of aromatic system ($1630, 1610, 1590\text{ cm}^{-1}$) have been revealed.

In ^1H NMR spectrum of studied compound 5 singlet signal (s., 1.30 and 1.40 ppm) attributed to 2 methyl group; quartet peak (3.20 ppm, $J_1=4.14, J_2=6.43\text{ Hz}$) attributed to proton bound with carbon atom of epoxy cycle; 2 quartet each with area of 1H (4.40 and 4.60 ppm, $J_1=4.14, J_2=11.03\text{ Hz}$) attributed to oxygen-bound methylene group ($-\text{CH}_2-$). These signals provide an opportunity to confirm that simple side ethereal chain as



-group is present in the structure of studied compound. The signals detected in in lower magnetic field of spectrum: doublets each 1H area (6.25, $J=9.50\text{ Hz}$ and 8.15 ppm, $J=9.50\text{ Hz}$; 6.95, $J=2.10\text{ Hz}$ and 7.60 ppm, $J=2.30\text{ Hz}$) and singlet (7.15 ppm) were attributed to protons in positions of C-3, C-4, C-2', C-3' and C-8 of the furocoumarin structure.

The results obtained from IR- and ^1H NMR-spectra proves that a structure of compound-5 is identical with structure of oxypeucedanin.

Compound-6. The elemental composition and melting point of the compound obtained in individual state from fractions of 129-130 of the chromatographic column eluted by mixture of benzole and chloroform (1:3) and chloroform were $C_{16}H_{16}O_6$, m.p. 137.0-138.0°C, respectively.

In the area of characteristic IR-spectrum absorption frequencies the bands characterizing of hydroxyl group (3400 cm^{-1}), $\text{C}=\text{O}$ group of δ -lactone cycle (1703 cm^{-1}) and double bonds of aromatic system ($1618, 1603, 1575, 1554\text{ cm}^{-1}$) are present.

In the ^1H NMR spectrum of the compound the signals relating to 2 methyl groups (s., 1.30 and 1.40 ppm), 2 hydroxyl groups (s., 2.35 and 3.05 ppm), to protons of methylene (t., 4.45 and 4.55 ppm) and gem-hydroxyl (d., 3.90 ppm) groups were detected.

The signals detected in in lower magnetic field of ^1H NMR spectrum: 6.30 (d., $J=9.65\text{ Hz}$, 1H, H-3),

8.20 (d., $J=9.65$ Hz, 1H, H-4), 7.30 (s., 1H, H-8), 7.00 (d., $J=2.30$ Hz, 1H, H-3'), 7.60 m.h. (d., $J=2.30$ Hz, 1H, H-2') characterize furocoumarin part of the molecule. In ^{13}C NMR spectrum of studied compound providing the presence of 16 carbon atoms in molecule 16 singlet signals (25.0; 27.0; 30.0; 71.0; 74.0; 94.0; 99.0; 104.0; 108.0; 111.5; 112.0; 119.0; 133.0; 139.0; 145.0; 161.0 ppm) were detected.

Seven signals that were not detected in ^{13}C Dept 135 spectrum prove that the number of non-protonated carbon atoms is seven. Based on above-mentioned spectral data it is proved that the structure of compound-6 is identical to structure of oxy-peucedanin hydrate.

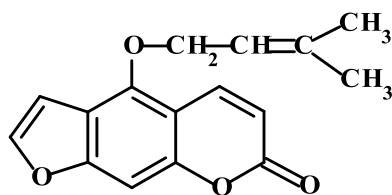
Compound-7. The elemental composition and melting point of the crystalline compound obtained in individual state from fractions of 129-131 of the chromatographic column eluted by mixture of chloroform and ethanol (95:5) were $\text{C}_{11}\text{H}_6\text{O}_4$, m.p. 286.0-289.5°C, respectively.

In the IR-spectrum the bands characterizing of hydroxyl group (3226 cm^{-1}), C=O group of δ -lactone cycle (1690 cm^{-1}) and double bonds of aromatic system (1585 , 1253 , 824 cm^{-1}) were detected. These bands allow attributing studied compound to simple linear furocoumarins (Li et al., 2006; Ghada et al., 2015).

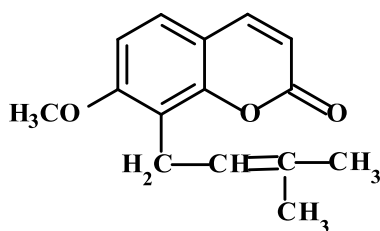
In ^1H NMR spectrum the signals were detected each with area of 1 proton unit: 6.19 (1H, d., $J=9.90$ Hz, H-3), 8.25 (1H, d., $J=9.90$ Hz, H-4), 11.10 (1H, HO-), 6.90 (1H, s., H-8), 7.76 (1H, d., $J=2.40$ Hz, H-2') and 7.48 ppm (1H, d., $J=2.40$ Hz, H-3').

The interpretation of IR- and ^1H NMR-spectral data prove that the structure of studied compound is identical to the structure of oxy-furocoumarin bergaptol.

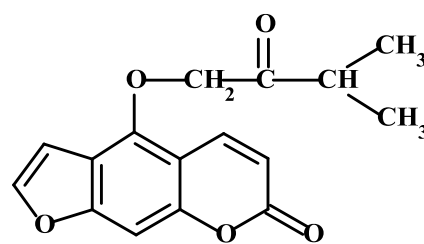
The chemical structure of compounds identified as a result of researches:



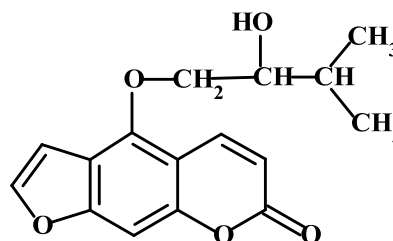
Isoimperatorin



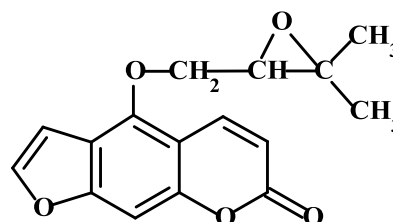
Osthol



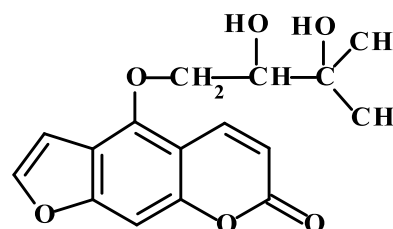
Isooxyypeucedanin



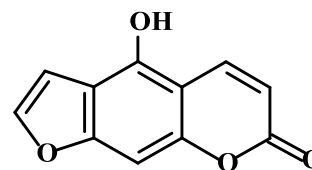
Pranferol



Oxypeucedanin



Oxypeucedanin hydrate



Bergaptol

CONCLUSIONS

1. From the roots of *Prangos biebersteinii* Karjag. collected in flowering phase from Beshbar-mag mountain 7 coumarin derivatives in individual state have been obtained.

2. Based on physicochemical (elemental composition, melting temperature) properties and spectral (IR- and NMR) data the obtained individual coumarin derivatives were identified as izoimperatorin, ostol, izooxyypeucedanin, pranferol, oxy-peucedanin, oxypeucedanin hydrate and bergaptol.

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Prangos biebersteinii Karjag. Növünün Köklərinin Bioloji Fəal Maddələrinin Öyrənilməsi

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AMEA Botanika İnstitutu

Prangos biebersteinii Karjag. (Biberşteyn çəşiri) növü köklərindən alınmış ekstraktiv maddələr cəmindən sütunlu xromatografiya metodundan istifadə edərək fərdi şəkildə 7 kristallik maddə alınmışdır: C₁₆H₁₄O₄, ə.t. 108,0-109,0°C (1), C₁₅H₁₆O₃, ə.t. 84,0-85,0°C (2), C₁₆H₁₄O₅, ə.t. 145,0-146,0°C (3), C₁₆H₁₆O₅, ə.t. 109,0-110,0°C (4), C₁₆H₁₄O₅, ə.t. 141,0-143,0°C (5), C₁₆H₁₆O₆, ə.t. 137,0-138,5°C (6) və C₁₁H₆O₄, ə.t. 286,0-289,5°C (7). Alınmış maddələrin fiziki-kimyəvi xassələrinin (tərkibi, ə.t.) və spektral (İQ- və NMR-) xüsusiyyətlərinin tədqiq edilməsindən alınan nəticələr əsasında onlar uyğun olaraq izoimperatorinlə (1), ostolla (2), izooksipepsedaninlə (3), pranferolla (4), oksipepsedaninlə (5), oksipepsedanin hidratla (6), berqaptolla (7) identifikasiya edilmişdir.

Açar sözlər: *Prangos*, köklər, maddələr cəmi, xromatografiya, fərdi, İQ spektroskopiyası, NMR spektroskopiyası, kimyəvi sürüclər

Изучение Биологически Активных Веществ Корней *Prangos biebersteinii* Karjag.

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Институт ботаники НАН Азербайджана

Исследованы корни *Prangos biebersteinii* Karjag., собранные на горе Бешбармак. Из ацетонового экстракта корней *P. biebersteinii* методом колоночной хроматографии (Al_2O_3 , III-IV степени активности) выделены 5 кристаллических веществ кумариновой природы: $\text{C}_{16}\text{H}_{14}\text{O}_4$, т.пл. 108,0-109,0°C (1), $\text{C}_{15}\text{H}_{16}\text{O}_3$, т.пл. 84,0-85,0°C (2), $\text{C}_{16}\text{H}_{14}\text{O}_5$, т.пл. 145,0-146,0°C (3), $\text{C}_{16}\text{H}_{16}\text{O}_5$, т.пл. 109,0-110,0°C (4), $\text{C}_{16}\text{H}_{14}\text{O}_5$, т.пл. 141,0-143,0°C (5), $\text{C}_{16}\text{H}_{16}\text{O}_6$, т.пл. 137,0-138,5°C (6), $\text{C}_{11}\text{H}_6\text{O}_4$, т.пл. 286,0-289,5°C (7), которые на основании физико-химических свойств (элементный состав, температура плавления) и спектральных (ИК- и ЯМР-спектры) данных идентифицированы, соответственно, с изоимператорином (1), остолом (2), изооксипейцеданином (3), пранферолом (4), оксипейцеданином (5), оксипейцеданин гидратом (6) и бергаптолом (7).

Ключевые слова: *Прангос, корни, сумма веществ, хроматография, индивидуальный, ИК-спектроскопия, ЯМР-спектроскопия, химический сдвиг.*

New Host Records of the Genus *Golovinomyces* (Erysiphales, Ascomycota)

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Samples of *Polygonum alpinum* and *Alcea rosea* infected by powdery mildews collected from Azerbaijan were analyzed by using morphological and molecular methods. 28S rRNA gene including D1/D2 domains and ITS1/5.8S/ITS2 regions were determined for each specimen. Consequently, *Golovinomyces spadiceus* on *P. alpinum* and *G. magnicellulatus* on *A. rosea* were identified. Comprehensive morphological descriptions, illustrations of species and the results of molecular-phylogenetic analysis were given in the present article.

Keywords: Erysiphaceae, molecular analysis, new host plant, powdery mildew fungi, taxonomy

INTRODUCTION

Powdery mildew fungi (Erysiphaceae) are economically important plant pathogens of vascular plants, including many cultivated plants. This group of fungi is characterized by their obligate biotrophic nature. Powdery mildews have long been considered as strictly host specific, in which host range of single powdery mildew species is restricted to a single plant family, or narrow range of genera or species (Matsuda, Takamatsu, 2003; Glawe, 2008; Braun, Cook, 2012). This assumption was supported with molecular analyses in many cases. But there are some exceptions, in which isolates having identical or highly similar DNA sequences are found from many distantly related host plants. For example, *Golovinomyces orontii* (Castagne) Heluta, *Leveillula taurica* (Lév.) G. Arnaud and *Podospheera xanthii* (Castagne) U. Braun & Shishkoff. are strictly herb parasitic fungi and have wide range of host plants beyond family level. In comparison with tree-parasitic powdery mildews having older origin and narrow range of hosts, herb parasitic powdery mildews have recent origin and considered that they are continuing their host expansion (Khodaparast et al., 2001; Matsuda, Takamatsu, 2003; Takamatsu et al., 2013). Exclusively tree-parasitic powdery mildew fungus, *Phyllactinia guttata* (Wallr: Fr.) Lév. represents as a species complex composed of numerous species. Some tree parasitic species, such as *Erysiphe alphitoides* (Griff. & Maubl.) U. Braun & S. Takam., *E. platani* (Howe) U. Braun & S. Takam., and *E. quercicola* S. Takam. & U. Braun are also considered to be actively expanding their host ranges to numerous unrelated host genera and families other than their original hosts (Kirschner, Liu, 2014; Takamatsu et al., 2015; Siahaan et al., 2016). Recently, 11 tropical tree gene-

ra were listed as hosts for *E. quercicola* (Limkainsang et al., 2006). Sequences identical to *E. alphitoides* on herbaceous *Oenothera* spp. were determined by molecular analysis, which was the first record of this fungus on herbaceous plants (Bereczky et al., 2015). *E. platani* expanded its host range to Simaroubaceae (*Ailanthus altissima*), beyond its original host Platanaceae family (Beenken, 2017).

Studies of powdery mildews in Azerbaijan began in mid of the last century, when numerous taxa from Erysiphales were recorded during exploring of mycobiota in this country (Мехтиева, 1956; Ключева, 1965; Ахундов, Агаева, 1978; Ахундов, 1979). However, taxonomic literature has significantly changed during last 20 years. Comprehensive investigation is required to clarify and renovate the knowledge about this group of fungi by application of modern morphological and molecular methods with additional collections.

During our study, we found powdery mildews on *Polygonum alpinum* All. (Polygonaceae) and *Alcea rosea* L. (Malvaceae) collected in 2015–2016 from Baku, Azerbaijan. Both host species are herbaceous plants, of which *Alcea* L. species are grown as an ornamental plant. Based on the morphological examinations, catenate conidia and nipple shaped appressoria were found, suggesting that the fungi from our specimens belong to the genus *Golovinomyces*. Powdery mildews from this genus infect up to 2283 plant species from 58 families worldwide and have never been recorded on the Polygonaceae family (Amano, 1986). Two *Golovinomyces* species, *G. americanus* and *G. orontii* were recorded on some plant species from Malvaceae family, of which *G. americanus* is known to be distributed only in North America (Braun, Cook, 2012). *G. spadiceus* on *P. alpinum*

and *G. magnicellulatus* on *A. rosea* were identified based on molecular and morphological examinations of our specimens. Thus, morphological description, illustration of species and results of molecular-phylogenetic analysis were given in the present study.

MATERIALS AND METHODS

Samples used in this study were deposited in Mycological Herbarium of Mie University (TSU-MUMH, Tsu, Japan) and Mycological Herbarium of Institute of Botany, Azerbaijan National Academy of Sciences (BAK, Mycological Herbarium, Baku, Azerbaijan). Collection date, location, host plant species and accession numbers of the nucleotide sequences were given.

Morphological examination. For observation of asexual morph on the dried herbarium samples, lactic acid was used according to the procedure described by Shin & La (1993). Little piece of infected leaf was mounted in lactic acid on microscopic slide and gently boiled, then asexual structures were scrapped off from the leaf surface and observed under the optical microscope (Axio Imager, Carl Zeiss, Göttingen, Germany) with phase contrast using 10×, 20× and 40× objectives. Thirty conidia and conidiophores were measured for each specimen. The original size of conidia reconstructed with Bulmer's factor (Braun, Cook, 2012). Drawings were carried out by freehand using scale bar.

Molecular and phylogenetic analysis. DNA was extracted from mycelia by the chelex method (Hirata, Takamatsu, 1996). The ITS1/5.8S/ITS2 regions and 5'-end of the 28S rDNA gene (including D1 and D2 domains) were amplified separately by single polymerase chain reaction (PCR) (Meeboon, Takamatsu, 2015). PCR reaction was conducted with TaKaRa PCR thermal cycler Dice (TaKaRa, Tokyo, Japan). A negative control lacking DNA template was included for reactions. Primer sets ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3')/ PM6G (5'-CGAGCCCCAACACCAA-3'), and PM5G (5'-GACCCTCCACCCGTGT-3')/ NLP2 (5'-GGTCCCAACAGCTATGCTCT-3') were used for amplification. PM6G and PM5G are specific primers for *Golovinomyces* species. The PCR products were subjected to electrophoresis in 1.5% agarose gel in TBE buffer, then stained with Midori Green Advance DNA stain (Nippon Genetics Europe GmbH, Germany) and visualized under UV light. DNA samples were sent to SolGent (Daejeon, South Korea) for sequencing.

Two newly obtained sequences determined in this study were deposited to DNA Data Base of Japan (DDBJ) under the accession numbers LC331790 and LC331791. These sequences were aligned with other closely related sequences of the Erysiphales retrieved from GenBank using MUSCLE implemented in MEGA 7 (Edgar, 2004; Kumar et al., 2016). Phylogenetic tree was constructed using maximum parsimony (MP) method implemented in PAUP 4.0a157 (Swofford, 2002). MP analysis was run using heuristic search option with tree bisection reconnection (TBR) algorithm with 100 random sequence additions to find global optimum tree. The strength of internal branches in resulting tree was tested with bootstrap (BS) analysis using 1000 replications with step-wise addition option set as simple (Felsenstein, 1985). BS values of 70% or higher were given on the representative branch (Figure 1). Tree scores, including tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

RESULTS AND DISCUSSION

Phylogenetic analysis. Two sequences of ITS regions and 28S rRNA gene were determined from specimens on *P. alpinum* and *A. rosea* and aligned with closely related 21 sequences from the genus *Golovinomyces*. *Arthrocladiella mougeottii* (Lév.) Vassilkov (AB329690) was used as outgroup (Takamatsu et al., 2013). Totally the data matrix consisted of 24 sequences and 1272 characters, of which 1134 (89.5%) of them were constant and 58 (4.6%) characters were variable and parsimony-uninformative. Only 80 (6.3%) characters were informative for parsimony analysis. Subsequently, 518 equally parsimonious trees with 207 steps were constructed by the MP analysis. The best tree was chosen by Kishino-Hasegawa (Kishino, Hasegawa, 1989) and Shimodaira-Hasegawa (Shimodaira, Hasegawa, 1999) topology tests and a tree with the highest likelihood value was shown in Figure 1.

Taxonomy. Conidia producing in chains (catenate), *Euoidium* type of conidial germination and nipple shaped appressoria indicated that the fungi from our specimens belong to the genus *Golovinomyces*. Only morphology of asexual structures is not enough to identify powdery mildews in species level. Therefore, identification of species was done based on combined results of molecular and morphological analysis.

Golovinomyces spadiceus (Berk. & M.A. Curtis) U.Braun, Braun & Cook 329, 2012. (Fig. 2, 3).

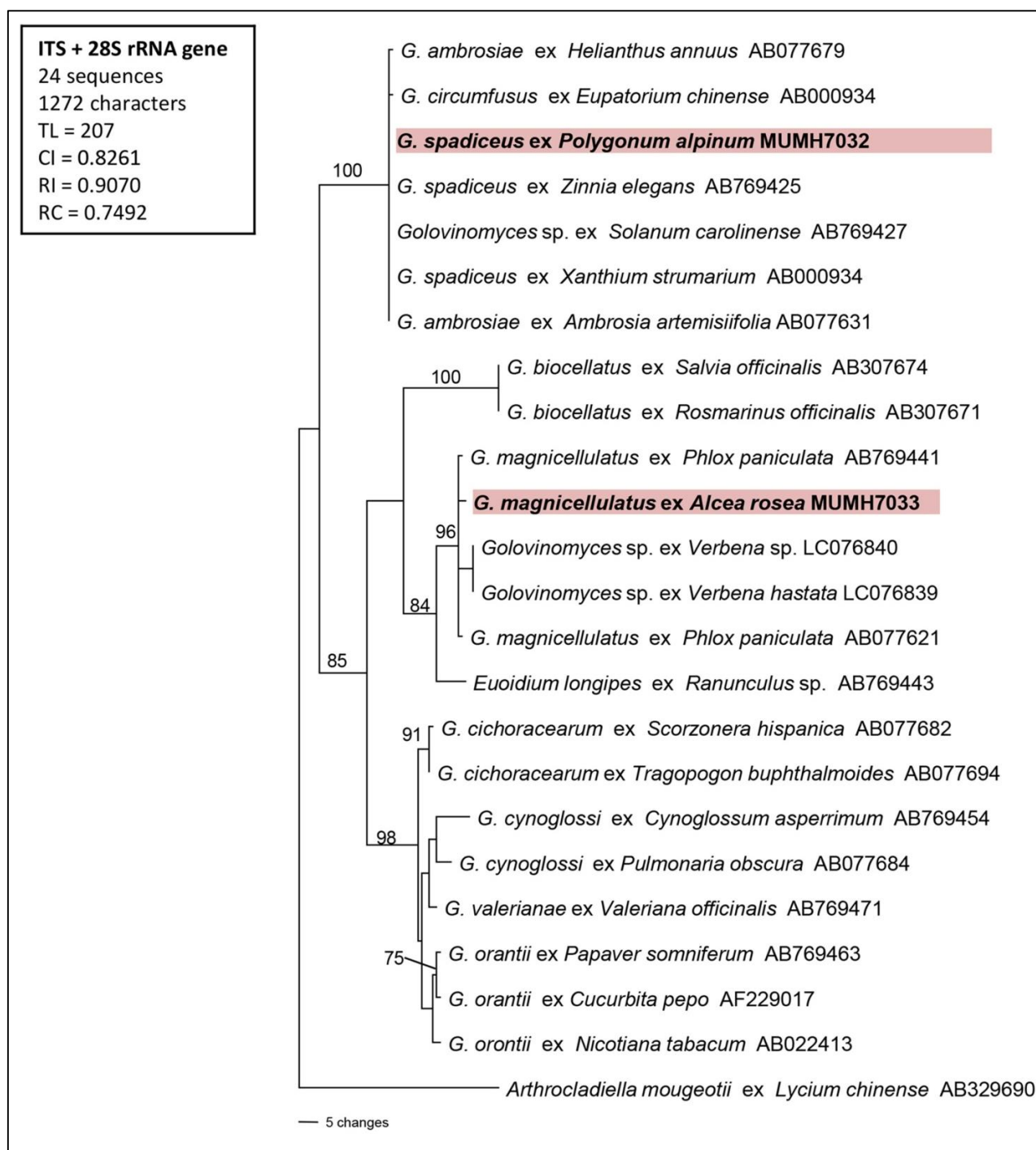


Figure 1. Phylogenetic tree of the genus *Golovinomyces* inferred from the ITS + 28S rRNA gene sequences constructed by maximum parsimony (MP) method. Bootstrap (BS) values ($\geq 70\%$) were given on the branches. Sequences determined in this study were highlighted.

Description: Mycelium amphigenous, in dense, effuse or in irregular patches, sometimes covers entire leaf surface, white to grayish, persistent. Hyphae 3–7 μm diam., thin-walled, hyaline, smooth, hyphal appressoria nipple-shaped, solitary. Conidiophores arising from the upper surface of mother cells and towards one end of cell, up to 135 μm long. Foot-cells cylindrical, straight, 20–50 \times 9–12 μm , followed by 1–3 shorter cells. Conidia formed in chains, ellipsoid, obovoid, doliiform, 26–41 \times 16–23

μm , length/width ratio 1.4–2.0. Conidial germination is in the *Euoidium* type, germ tubes arising lateral or slightly medium, with swollen appressorium on end.

Material examined: On *Polygonum alpinum* All. (Polygonaceae), Lokbatan, Baku, 08 Sept. 2015, leg. L.V. Abasova, BAK Mycological Herbarium No 10057, TSU-MUMH 7032, DDBJ ID number: LC331790 (ITS and 28S rRNA gene).

Remarks: *Leveillula taurica* and *Erysiphe polygoni* DC. have been described on *Polygonum*

species worldwide (Braun, Cook, 2012). Determined nucleotide sequences of the 28S rRNA gene and ITS regions from our specimen were completely identical to the sequences of *Golovinomyces spadiceus* (AB769425) on *Zinnia elegans* in GenBank. Three *Golovinomyces* species, *G. ambrosiae*, *G. spadiceus* and *G. circumfusus* listed in manual (Braun, Cook, 2012), mainly occur on the plants from the tribe Heliantheae of Asteraceae. Takamatsu et al. (2013) reported that isolates of these three species are homogenous and placed in the same clade and differentiation of these species on genetic level is difficult. So, it is not reliable to identify species only based on molecular results, because there are some allied taxa with closely related nucleotide sequence data (Takamatsu et al., 2013). In the present phylogenetic analysis (Figure 1), the sequence from our specimen placed in the clade with *G. ambrosiae*, *G. circumfusus*, and *G. spadiceus*, supported by 100% BS value in MP analysis. We checked the nucleotide similarity among these species. *G. ambrosiae* and *G. circumfusus* were 99.9% similar (only one base differences per each species) to our specimen in the ITS region. But there are two substitutions difference between *G. ambrosiae* and our specimen. However, some morphological differences among these species were reported. *G. circumfusus* has longer (30–110 µm) and straight to curved foot-cells, whereas foot-cells in *G. spadiceus* (30–80 µm) are shorter and straight. Limoniform shape of conidia was recorded in both, *G. circumfusus* and *G. ambrosiae*, but not in *G. spadiceus*. Length/width ratio in *G. ambrosiae* (1.4–1.6) is smaller than *G. spadiceus* (1.5–2), but in *G. circumfusus* (1.3–2.6) is bigger (Braun, Cook, 2012). Thus, our specimen was identified as *G. spadiceus* based on morphological and molecular data. This is the first record of the genus *Golovinomyces* on Polygonaceae family in the world. *P. alpinum* is a new host record for *G. spadiceus*.



Figure 2. *Golovinomyces spadiceus* on *Polygonum alpinum* (MUMH 7032; MH No 10057). A. Conidiophore; B. Germinated Conidia; C, D. Conidia; Scale bar = 30 µm.

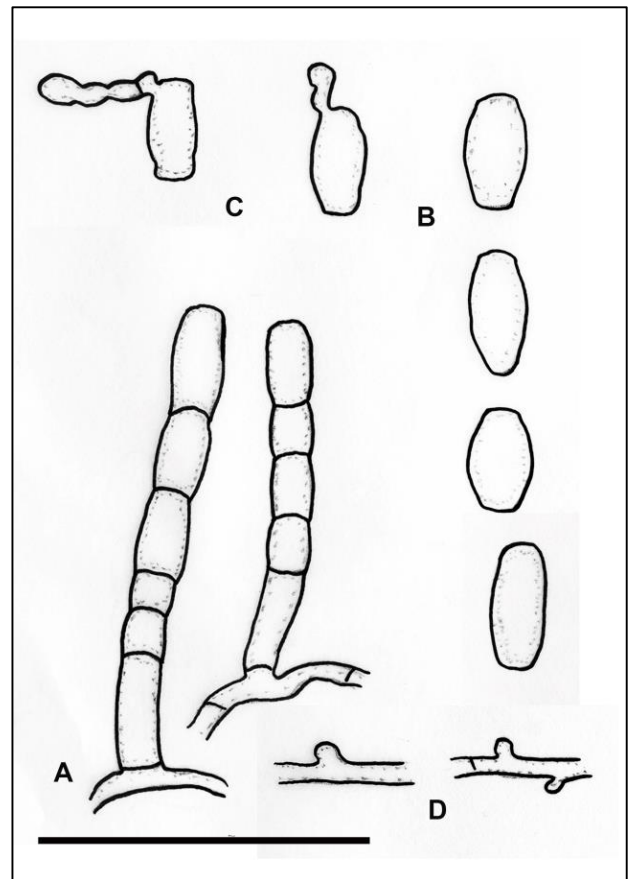


Figure 3. Asexual morphs of *Golovinomyces spadiceus* on *Polygonum alpinum* (MUMH 7032; MH No 10057). A. Conidiophores; B. Conidia; C. Germinated conidia; D. Hyphal appressoria; Scale bar = 100 µm.

Golovinomyces magnicellulatus (U. Braun) Heluta, Ukrayins'k Bot. Zhurn. 45 (5): 63, 1988 (Fig. 4, 5).

Description: Mycelium amphigenous, thin, effuse, covers entire leaf surface, grayish, evanescent to persistent. Hyphae thin-walled, hyaline, smooth, 4–7 µm diam., hyphal appressoria nipple-shaped, solitary. Conidiophores arising from upper surface of mother cells, towards one end of cell, 140–270 µm long. Foot-cells cylindrical, straight, (45–)58–130 × 9–12 µm, followed by 1–3 shorter cells, thin from the base, becoming wide above. Conidia formed in chains, ellipsoid, cylindrical to doliiform, (29–)31–37 × 16–19 µm, length/width ratio (1.3–)1.7–2.2, conidial germ tubes arising terminal, short and with swollen appressorium, *Euoidium* type.

Material examined: On *Alcea rosea* L. (Malvaceae), Botanical garden, Baku, 12 May 2016, leg. L.V. Abasova, BAK Mycological Herbarium No 10058, TSU-MUMH 7033, DDBJ ID number: LC331791 (ITS and 28S rRNA gene).

Remarks: *Alcea* species are biennial or perennial herbs and cultivated as an ornamental plant. *Leveillula contractirostris* Heluta & Simonyan was reported as powdery mildew pathogen on *Alcea*

species (Braun, Cook, 2012). However, molecular and morphological results indicated that our specimen belongs to the genus *Golovinomyces*.

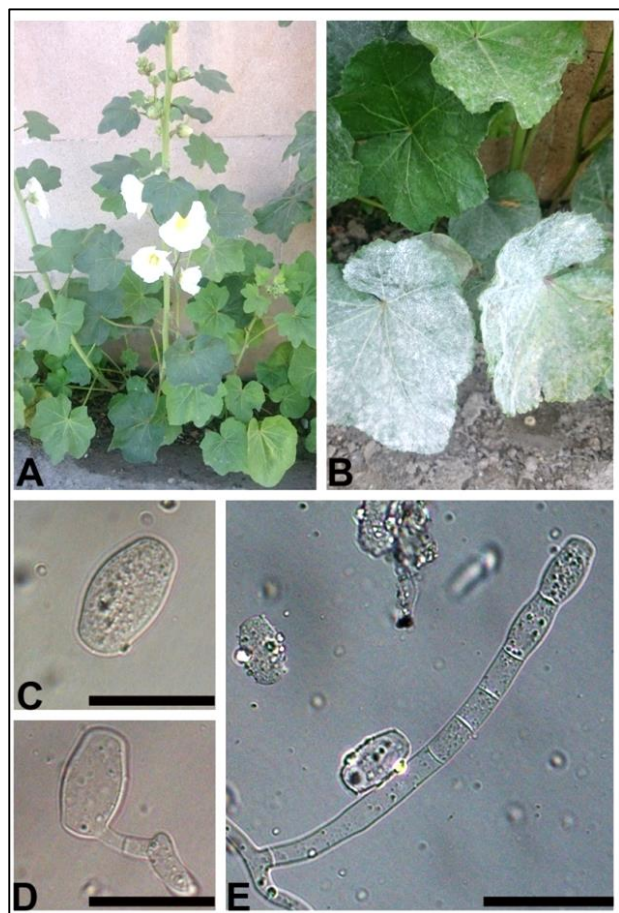


Figure 4. *Golovinomyces magnicellulatus* on *Alcea rosea* (MUMH 7033; MH No 10058). A. Plant; B. Disease symptoms; C. Conidia; D. Germinated conidia; E. Conidiophore; Scale bar = 50 μ m.

Sequences of ITS and 28S rRNA gene were 99% identical to the sequences of *G. magnicellulatus* (AB077621; AB769442) on *Phlox paniculata* and *G. orontii* (AB307670) on *Verbena hortensis* in GenBank. Nucleotide sequence similarity between *G. magnicellulatus* and our specimen was 99% (4 substitutions difference) in ITS region and 100% identical in 28S rRNA gene. Two varieties of *G. magnicellulatus* are known, and both of them were recorded on *Phlox* and *Polemonium* spp. (*Polemoniaceae*). Morphological differences between the two varieties is in width of chasmothecial appendages and peridium cells. In the meantime, *G. orontii* and *G. americanus* are recorded on some species from Malvaceae family. *G. americanus* is a powdery mildew fungus endemic to North America, whereas *G. orontii* is distributed worldwide.

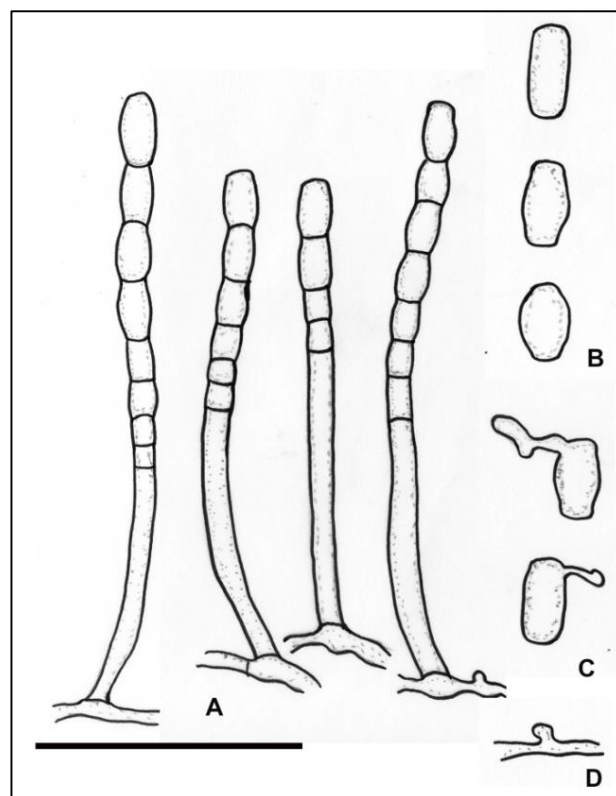


Figure 5. Asexual morphs of *Golovinomyces magnicellulatus* on *Alcea rosea* (MUMH 7033; MH No 10058). A. Conidiophores; B. Conidia; C. Germinated conidia; D. Hyphal appressoria; Scale bar = 100 μ m.

Asexual morphs of our specimen are more similar to *G. magnicellulatus* than *G. orontii*. Curved foot-cells, twisted or forked germ tubes in *G. orontii* is distinguishable characters from our specimen (Braun, Cook, 2012). In the phylogenetic analysis (Figure 1) sequence obtained from our specimen is located within the sequences of *G. magnicellulatus* and *Golovinomyces* sp. on *Verbena* and supported by 96% BS value in MP analysis. Our specimen was identified as *G. magnicellulatus* according to the molecular and morphological data. However, we could not distinguish the variety, because our specimen was in asexual stage.

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***Golovinomyces* (Erysiphales, Ascomycota) Cinsinə Aid Yeni Sahib Bitkilərə Dair Qeydlər**

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Azərbaycandan toplanmış unlu şəh göbələkləri ilə yoluxmuş *Polygonum alpinum* və *Alcea rosea* nümunələri morfoloji və molekulyar metodlarla tədqiq edilmişdir. Hər bir nümunə üçün 28S rRNT-nin D1/D2 domenləri daxil olmaqla, ITS1/5.8S/ITS2 sahələri, müəyyən edilmişdir. Nəticədə, *P. alpinum* üzərində *Golovinomyces spadiceus* və *A. rosea* üzərində *G. magnicellulatus* növləri təyin olunmuşdur. Məqalədə bu növlərin ətraflı morfoloji təsviri, illustrasiyası və molekulyar-filogenetik analizlərinin nəticələri verilmişdir.

Açar sözlər: *Erysiphaceae*, molekulyar analiz, yeni sahib bitki, unlu şəh göbələkləri, taksonomiya

Новые Данные О Растениях-Хозяевах Рода *Golovinomyces* (Erysiphales, Ascomycota)

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Исследованы образцы с *Polygonum alpinum* и *Alcea rosea*, зараженные мучнисто-росяными грибами, собранные в Азербайджане с использованием морфологических и молекулярных методов. Для каждого образца, включая домены Д1/Д2 28S рРНК, были определены зоны ITS1/5.8S/ITS2. В результате на *P. alpinum* был идентифицирован вид *Golovinomyces spadiceus* и на *A. rosea* - *G. magnicellulatus*. В данной статье приведены подробное морфологическое описание, иллюстрация видов и результаты молекулярно-филогенетического анализа.

Ключевые слова: *Erysiphaceae*, молекулярные анализы, новые растения-хозяева, мучнисто-росяные грибы, таксономия

Dynamics of the Accumulation of Flavonoids in *Polygonum L.* Species

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There are 300 species of *Polygonum L.* genus of *Polygonaceae* Juss. family in the temperate zone of the world. Some of them are medicinal plants, which are used for the treatment of diseases or as a raw material for preparing medicines. Flavonoids are active substances of the species belonging to the *Polygonum L.* genus. Flavonoid content, distribution, dynamics of accumulation throughout growth phases, productivity of above-ground parts have been studied in various organs of *P. aviculare* and *P. patulum L.* species. Uneven distribution of flavonoids was detected in plant organs. In generative organs maximum accumulation of flavonoids was observed in buds, while in vegetative organs it was observed in leaves. Maximum biomass productivity and flavonoid content were observed in the flowering phase of both species.

Keywords: *Polygonum*, flavonoid, dynamics of accumulation, vegetative, generative organs, biomass

INTRODUCTION

Owing to a wide range of biological activity of flavonoids, taxa rich in flavonoids are being searched and plants with known flavonoids are being studied more comprehensively. There were reports on anti-radiant, antispasmodic, antioxidant, antimutagenic and other properties of flavonoids (Baraboy, 1976; 1984; Mashkovskiy 2005, Cook and Samman, 1996; Tijburg et al., 1997; Dicarbo et al., 1999). The ability to decrease conductivity of capillaries and increase their elasticity are main biological properties of flavonoids and other polyphenols of plant origin. Recently, antioxidant properties providing the protection from free radicals as stress factors causing pathological changes in the human organism have been extensively studied (Rice-Evans and Müller, 1996; Kang and Kapoor, 2002). Plants containing flavonoids are considered to be a valuable raw material for medications, such as capillary strengtheners, immunomodulators, anticancerogenes and cholagogues.

One of the taxa containing flavonoids is the species of *Polygonum L.* genus of the *Polygonaceae* Juss. family. 300 species of the *Polygonum L.* genus of the *Polygonaceae* Juss. family are known to spread in the temperate zone of the world (Svelyev, 1996; Wang et al., 2005). Most of them are grown as decorative, technical plants and fodder (Svelyev, 1996). *Polygonum* species are used in the traditional medicine of many nations in the treatment of cardiovascular diseases (Yim et al., 2000), as an anti-inflammatory agent (Bralley et al., 2008), neuroprotector (Wang et al., 2009), age-related Alzheimer (Um et al., 2006), Parkinson (Li et al., 2005) diseases.

Various species of the genus, especially *P. aviculare L.* included in the pharmacopeia of many countries are used as a raw material for developing medical preparations. As they are rich in polyphenols of plant origin, especially flavonoids. One of the tasks of scientific and practical importance when using a plant as a raw material is to determine in which organ of the plant and during which phase of the growth active substances are accumulated as much as possible.

The main purpose of the work was to determine the spread of flavonoids in plant (*P. aviculare L.* and *P. patulum L.*) organs, and the pattern of accumulation depending on the growth phases, biomass productivity, flavonoid yield and optimal time period for collecting a plant raw material.

MATERIALS AND METHODS

Various organs of *P. aviculare L.* and *P. patulum L.* species were used as plant materials. Plant materials were collected at the beginning of vegetation, during the periods of the development of buds, before flowering (25-35% of buds were mature), flowering and fruit development in the botanical-geographical region of the Guba region of Greater Caucasus, in the north-eastern part of the Urva village of the Gusar region, around the forest and in the Susay village of the Guba region in 2014-2016.

Whole model plants were taken in various phases of the development. Roots were cleaned, plants were weighed and dried. Vegetative (leaf, stem, root) and generative (bud, flower, fruit) organs of each plant were detached and prepared

for analysis. Analysis was always performed with the material of the same population. Productivity of the above-ground part was established. Fresh and dry weights of plants collected from 4x1 m² field separated from the 100m² field were found. The density of *P. aviculare* and *P. patulum* species in 1 m² field was 15 plants and 16 plants, respectively. For the analysis of the raw material, plants were dried in the air and cut into 1mm slices. Amount of dry matter was determined using MX-50 moisture analyzer. To found dynamics of the changes in the flavonoid content and productivity of the above-ground part, 30 plants were taken for each sample. Sum of the flavanoids was determined in various plant organs spectrophotometrically (Gosfar 1989). Total amount of flavonoids was estimated based on avicularin according to the following formula:

$$x = \frac{D * 100 * 100 * 25}{330 * m * (100 - w)}$$

D – optic density; 330 - absorption index of avicularin with AlCl₃ at 410 nm; *m*-mass of the material, *w*- lost weight upon drying (%).

Each analysis was performed in 3 replicates and the mean values were estimated. The relative error of the methodology was ± 1.4%.

RESULTS AND DISCUSSION

The study of the accumulation dynamics of flavonoids in various organs of *P. aviculare* and *P. patulum* species belonging to the *Polygonum* L. genus depending on growth phases showed pronounced changes in flavonoid contents during the vegetative period (Table 1). In generative organs of *P. aviculare* and *P. patulum* species maximum amounts of flavonoids were accumulated in flowers (7.11 and 5.7%, respectively), and minimum amounts in green fruit (1.91 and 2.83%, respectively). In vegetative organs maximum amounts were found in leaves (4.85; 4.44%) and minimum amounts in roots (0.41 and 0.31%, respectively) and stem (0.71-0.65%, respectively).

In both species flavonoid content was found to change sharply depending on the growth stage of the plant. Maximum flavonoids (4.85%) were accumulated in leaves of the *P. aviculare* L. species before flowering, while in *P. patulum* L. maximum accumulation (4.44%) occurred during the flowering phase.

In both species flavonoid contents in the leaves of the studied species were found to be maximum in the phase of fruit development (2.83 and 3.03, respectively). Compared to other vegetative organs minimum flavonoids were accumulated in roots. In roots of *P. aviculare* L. and *P. patulum* L. maximum flavonoids were accumulated during the de-

velopment of buds (0.82%), and before flowering (0.67%), respectively. In above-ground organs of the studied species minimum flavonoids were detected in stems. Maximum flavonoids (1.34%) were found in the stem of *P. aviculare* L. during the initial phase of vegetation. But in *P. patulum* L. maximum amount was observed in the phase of bud development (1.13%), and minimum amount in the fruit development (0.71%) phase and the initial vegetation period (0.65%).

Flavonoid contents in the above-ground organs of the studied species changed in the range of 2.21-3.81%. In above-ground parts of *P. aviculare* L. maximum flavonoid contents were found during mass-flowering period (3.81%), while in *P. patulum* L. the highest quantity was detected before flowering (4.08%).

The study of the productivity of plant parts used as raw materials is of great practical importance. *P. aviculare* L. is a known medicinal plant. The study of the flavonoid content in the above-ground parts of *P. patulum* L. confirmed that the flavonoid content of this plant met the demands of pharmacopeia and could be used as a raw material. Therefore, dynamics of the increase in above-ground mass of both species has been studied throughout phenological phases. Data on biomass productivity are presented in Table 2. The results show that depending on the growth phases both fresh and dry mass production of the plant changed in a large range. So, in *P. aviculare* L. fresh and dry masses changed in the ranges of 2.85-25.0g and 0.31-4.62 g, respectively. While in *P. patulum* L. these changes were 2.23-28.49 g and 0.25-5.44 g, respectively.

Maximum weight per a plant for *P. aviculare* L. based on fresh weight was found during mass-flowering (24.7 g) and based on dry weight during the development of fruit (5.12g). While in *P. patulum* L. the same values were 26.3 and 5.39 g, respectively. The obtained results showed that it was advisable to collect plant materials when using both species as raw materials during mass-flowering. But the optimum period of plant collecting should be established to produce flavonoids in a high quantity. This was established based on the plant density per a certain field (1 m²) and flavonoid amounts in plant mass in 100 m² field.

According to Tables 1 and 2 productivity of flavonoids in 100 m² and 1ha was calculated and the results were presented in Table 3. As seen in the Table maximum amounts of flavonoids from above-ground parts of both species can be obtained during the complete flowering phase (240 g/100 m² and 335.9 g/100 m²). The obtained results confirmed the practical importance of the studied species and showed that both species can be used as raw materials for producing flavonoids.

Table 1. Dynamics of the accumulation of flavonoids in various organs of the *Polygonum* species throughout growth phases (based on dry weight, %)

Analyzed organ	Years	Phases of growth				
		Begining of vegetation	Bud development	Before flowering	Mass-flowering	Fruit development
<i>P. aviculare</i> L.						
Stem	2014	1.15	0.99	0.96	0.71	0.61
	2015	1.53	1.32	1.28	0.94	0.82
	2016	1.31	1.16	1.13	0.83	0.71
	orta	1.34	1.21	1.12	0.82	0.71
Leaf	2014	2.75	4.06	4.15	3.70	2.45
	2015	3.66	5.32	5.53	4.93	3.27
	2016	3.22	4.44	4.87	4.34	2,77
	orta	3.21	4.61	4.85	4.32	2.83
Generative organ	2014	-	5.66	6,08	5.55	1.57
	2015	-	7.55	8,11	7.40	2,35
	2016	-	6.64	7.14	6.51	1.83
	orta	-	6.62	7,11	6.48	1.91
Above-ground part	2014	2.07	2.55	3.38	3.26	1.92
	2015	2.95	3.45	4.17	4.35	2.55
	2016	2.60	3,09	3.67	3.83	2.24
	orta	2.54	3.02	3.74	3.81	2.23
Root	2014	0.49	0.72	0.63	0.59	0.37
	2015	0.65	0.95	0.84	0.78	0.45
	2016	0.55	0.80	0.71	0.66	0.40
	orta	0.56	0.82	0.72	0.68	0.41
<i>P. patulum</i> L.						
Stem	2014	0.74	0.98	0.91	0.84	0.56
	2015	0.98	1.31	1.21	1.12	0.75
	2016	0.82	1.16	1.02	0.94	0.63
	orta	0.85	1.13	1.05	0.97	0.65
Leaf	2014	2.93	3.51	3.62	3.85	2.65
	2015	3.91	4.68	4.83	5.14	3.51
	2016	3.29	3.83	4.06	4.32	2.93
	orta	3.38	4.00	4.17	4.44	3.03
Generative organ	2014	-	4.27	4,85	4.48	2.46
	2015	-	5.58	6.47	5.88	3.28
	2016	-	4.67	5.43	5.02	2.76
	orta	-	4.84	5,58	5.13	2.83
Above-ground part	2014	1.93	2.90	3.37	3.66	2.14
	2015	2.54	3.96	4.69	4.48	2.85
	2016	2.16	3.38	3.97	3.75	2.51
	orta	2.21	3.41	4.08	3.86	2.50
Root	2014	0.34	0.46	0.58	0.37	0.27
	2015	0.46	0.55	0.79	0.49	0.36
	2016	0.39	0.47	0.65	0.41	0.30
	orta	0.39	0.49	0.67	0.42	0.31

Note: Deviation: $\pm 0.1 \div 0.2$ **Table 2.** Dynamics of the increase in the above-ground part of the *Polygonum L. species* (per a plant)

Species \ Phases	Beginning of vegetation	Bud development	Before flowering	Mass-flowering	Fruit development
<i>P. aviculare</i> L.					
Fresh weight	2.85	10.8	13.68	24.7	21.8
Dry weight	0.31	1.23	1.68	4.62	5.12
<i>P. patulum</i> L.					
Fresh weight	2.23	13.32	18.1	26.3	22.6
Dry weight	0.25	1.49	2.59	5.39	4.92

Table 3. Amounts of flavonoids obtained from *Polygonum* L. species during various phases of growth in 100 m² field. (mean values for 2014-2016)

Species	Phase of growth	Dry mass in 100 m ² , g	Flavonoid amount, g/100 m ²	Flavonoid amount (kg) in 1 ha
1	2	3	4	5
<i>P. aviculare</i> L.	Beginning of vegetation	465.0	11.81	1.18
	Bud development	1849.0	55.83	5.58
<i>P. aviculare</i> L.	Before flowering	2520.0	96.01	9.60
	Mass-flowering	16870	240.06	24.0
	Fruit development	6930.0	150.3	15.0
<i>P. patulum</i> L.	Beginning of vegetation	6739.0	8.84	0.88
	Bud development	2384.2	81.29	8.13
	Before flowering	4144.0	169.0	16.9
	Mass-flowering	8704.0	335.9	33.59
	Fruit development	8144.0	277.7	27.77

CONCLUSION

The study of the dynamics of the flavonoid accumulation in various organs of *P. aviculare* L. and *P. patulum* L. species of *Polygonum* L. genus showed that maximum accumulation occurred in reproductive organs (7.11-5.58%), less in leaves (4.85-4.44%) and minimum in roots (0.41-0.31%). Flavonoid contents were found to change sharply depending on the growth phase of the plant. Maximum flavonoids were accumulated in leaves of the *P. aviculare* species before flowering (4.85%), while in the *P. patulum* species it was observed during mass-flowering phase (4.44%). During the vegetative period the quantity of flavonoids changed in above-ground parts of the plants in the range of 2.21-3.84%. Maximum accumulation occurred in the *P. aviculare* L. and *P. patulum* L. species during the mass-flowering phase (3.81%), and before flowering (4.08%), respectively. Thus, the accumulation of flavonoids in plants is a dynamic process. The uneven distribution of flavonoids in plant organs and changes of their contents during various phases of the plant growth are related to their biological functions. The highest productivity of above-ground mass was detected during mass-flowering phase of both species (24.7 and 26.3 g/per a plant). It was established that both plants can be used as raw materials for obtaining flavonoids.

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***Polygonum* L. Növlərində Flavonoidlərin Toplanma Dinamikası**

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Qırxbuğumkimilər *Polygonaceae* Juss. fəsiləsinin qırxbuğum *Polygonum* L. cinsinin dünyanın mülayim qurşağında yayılmış 300 növü vardır. Onların bəziləri rəsmi dərman bitkisi olub, müxtəlif xəstəliklərin müalicəsində və dərman vasitələrinin alınmasında xammal kimi istifadə olunurlar. Cinsə daxil olan növlərin əsas təsiredici maddəsi flavonoidlərdir. Məqalədə *P. aviculare* və *P. patulum* L. növlərinin müxtəlif orqanlarında flavonoidlərin miqdarı, paylanması, inkişaf fazaları üzrə toplanma dinamikası, yerüstü hissə məhsuldarlığı öyrənilmişdir. Müəyyən edilmişdir ki, flavonoidlər orqanlar üzrə qeyri bərabər paylanır. Generativ orqanlardan maksimum miqdar qönçələrdə, vegetativ orqanlardan isə yarpaqlarda toplanır. Hər iki növün bioloji kütlə məhsuldarlığı və flavonoidlərin bitkiyə görə maksimum miqdarı çiçəkləmə fazasıdır.

Açar sözlər: *Polygonum*, flavonoid, toplanma dinamikası, vegetativ, generativ orqanlar, biokütlə

Динамика Накопления Флавоноидов У Видов Рода *Polygonum* L.

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В умеренном климатическом поясе обнаружено 300 видов рода *Polygonum* L. семейства *Polygonaceae* Juss. Некоторые из них являются лекарственными растениями, которые используются для лечения заболеваний или в качестве сырья для приготовления лекарств. Флавоноиды являются активными веществами вида, принадлежащего к роду *Polygonum* L. Содержание флавоноидов, их распределение и динамика накопления во всех фазах развития, а также продуктивность надземных частей изучались в различных органах видов *P. aviculare* и *P. patulum* L. Выявлено, что в различных органах растений распределение флавоноидов было неравномерным. Из генеративных органов максимальное накопление флавоноидов наблюдалось в бутонах, а из вегетативных органов - в листьях. Для обоих видов максимальные продуктивность биомассы и содержание флавоноидов отмечены в фазе цветения.

Ключевые слова: *Polygonum*, флавоноид, динамика накопления, вегетативные, генеративные органы, биомасса

About the Biological Diversity of Inland Water Ecosystems in Azerbaijan

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The article presents the number of species by groups registered by individual researchers in inland waters of the country (springs, rivers, reservoirs, lakes, etc.). Detailed information on the most common and dominant species in our water resources, their role in the ecosystem, etc. are provided. Since the beginning of scientific and zoological research, more than 1000 species of protozoan animals (mainly ciliated infusoria), more than 1350 species of invertebrate animals (mainly rotatory (more than 300 species) and insect larvae (more than 586 species)), 190 species of vertebrates [mainly birds (97 species) and fish (67 + 1 species) and 332 species of fish parasites (mainly myxosea (59 species), flatworms (69 species) and trematodes (81 species)] have been revealed. Indicating the species number of hydrobionts, will be changed as a result of future studies, since some groups of hydrabionts still remain unexplored, or only 1-2 species were accidentally recorded in the study. In addition to unicellular animals, cervical worms, nematodes, crustaceans, tusks, and other insect larvae can be included.

Keywords: *Water resources of Azerbaijan, biodiversity, Protozoa, invertebrates, vertebrates*

INTRODUCTION

Despite our country owns the least water resources among the countries of the South Caucasus, it is in a leading position in the region in terms of importance, wealth and study level of hidrofauna formed in its watercourses. It is likely due to the the extent of the countries territory, its natural wealth, landscape and climate diversity, availabilty of highly qualified national experts and their skills.

Also the richness of the rivers of Kura and Araz as well as the Caspian Sea are the most important factors contributing to this process. That is why since far from the past, Azerbaijan was in the loop of the various travelers (english, russian, french, german, dutch, etc) of the leading countries, and the similar sition occurs our days. Along with the underground and surface resources of Azerbaijan, the valuable living nature of the Caspian sea do consist of this interest's base.

There are more than 8350 small and large rivers on the territory of the country (Kura, Araz, Arpachay, Tartarchay, Hakichay, Lenkoranchay, Girdimançay, Ganix, Gabirry, Tala, Gangachay, Aghsuchay, Goychay, Astarachay, Mazımchay, Aghstafachay, Katexchay etc.), more than 800 lakes and confined water courses (Geygel, Maralgel, Garagel, Alagels', Aghgel, Mehman, the network of the Sarisu lakes, Hacıgabal, Mahmudchala, Aghchala, Acınohur, Jandar, Masazır, Mirzaladi,

Boyükhshor, Batabat lakes and confined water-courses etc.), formed ponds in the vicinity of the Kura and Araz (Aynalı, Garaoghlan, Yetim Kura, Marzli, Varvara, Ganikh, Mammadzamanlı etc.), more than 100 water reservoirs (Mingechaur, Shamkir, Varvara, Araz (Nakhchivan), Uzunoba, Nehram, Ashigbayramlı, Yekakhana, Arpachay, Aghstafachay, Shamkirchay, Tahtakorpu, Jeyranbatan, Khanbulanchay, Vilash, Behramtepe water junction etc.), more than 60km of irrigation canals (upper Shirvan, upper Garabagh highway canals etc.) and here is a drainage system, generating and a close the network.

The majority (7900) of our rivers are short (Rustamov, 1960) with lenth which less than 10km, when only 24 rivers are longer than 100km. Most of our small rivers are formed within the country territories, when the hugest and transboundary rivers (Kura and Araz and others) are formed and subjected to a strong human within the 3-5 countries those are crossing through and are.

The biggest parts of the Caucasus's largest rivers before they reach the Caspian sea are located within the Azerbaijany territory and are of the huge fishery importance. So, these rivers are the most valuable fish breeding and development locations of the Caspian.

The greatest lakes of the country (Aghgol, Sarysu lakes systems, Mehman, Hacıgabal, Mahmudchala, Mehman, Alagels, Gey-gel, Maralgel) and dozens small size lakes and ponds (Aynalı,

Garaoghlan, Marzli an others.) are the important fishery facilities.

Scaly fishes are habiting in the ponds and lakes, when also the caspian salmon and sturgeon fish species beside to the scaly species do exist in the downstream of the rivers directly connected with the Caspian sea.

The followings are the water reservoirs with the huge fishery importance and the significant watercourses forming the fish and water reservs of our country: Mingechaur water reservoir (WR) - built on the river Kura (functioning since 1953), Varvara wr (since 1956), Shamkir WR (since 1982), Araz WR (or Nakhchivan) built jointly with the government of Iran Islamic Republic on the river Araz (since 1972), Sarsang WR (since 1976) built on the Tartar river, Arpachay WR (since 1970) built on Arpachay river, Agstafachay WR (since 1970) built on the Agstafachay river, Ashygbayramly WR (since 1953) built on the Devebatan river, the water reservoirs Yekakhana and dozens of other small size ones (Kondalanchay, Nohurgishlaq, Javanshir, Khanbulanchay etc.).

Both of the natural (rivers, lakes, ponds etc.) and human made (water reservoirs, canals, swimming pools, artificial lakes, etc) are characterized with rich flora and fauna along with thier fish resources. In this respect, regardless of its size, strength and origin each water course is considered as standalone ecosystem.

The study of inland water ecosystems of the Republic is settled in a leading position among the countries of the Caucasus (not only the Southern Caucasus). More than 600 (some sources indicate 750) single-celled animal species and (mostly infuzorlar) and more than 1500 species of (data might vary by $\pm 1-3\%$) invertebrate animals are currently registered in the fresh water basins hydrofauna.

The key place (more than 500-600 species) belongs to Ciliata - free-living infuzors (Oligohymenophorea) as per living in the natural water basins and biodiversity. The following groups are *Rotatoria* (more than 200-300 species), *Chironomidae* (up to 150 species), *Cladocera* (up to 95-126 species), insects (124 species), the annelids (109 species), *Odonata* lavraes (more than 60 species), *Copepoda* (60 species), Molluscs (63 species), *Phryganeidae* (45 species), mayfly lavraes (40 species), *Plecoptera* lavraes (up to 32 species), other *Diptera* lavraes (more than 150 species), ostracodes (19 species), *Hirudinea* (15 species), *Anostraca* (5 species), isopods (3 species), triops (2 species), mysids (2 species) and *Tardigrada* (3 species) etc.

Among the listed main components of the water ecosystems the comparatively deeper inves-

tigated (i.e. with identified species composition) groups are *Oligohymenophorea* - free living infuzors (A.R.Aliev 1971-2012 years; I.Kh.Alekperov, 1972-2014 yeras), annelids (A.H. Gasimov, 1965), *Rotatoria* species (A.H.Gasimov, 1972), *Entomostraca* (A.N.Alizadeh, 1940, N.Talibov, 1965, I.Akhmedov 1971), *Anostraca* (A.R.Aliev, K.Tapdigova, 2014), *Chironomidae* (A.H.Gasimov, 1965, Z.Abdurrahmanova 1982).

Other groups of the particular importance - hirudineas, tardigradas, mayflies, trichopteras, plecopteras and others are still awaiting own investigators. That is why the special investigations towards this direction is one of the nearest future targets. And the investigation researchers still conducting research in this direction is and will be one of the main targets of the nearest future!

And now let us seperately review the groups which are attractive from the formation of biodiversity and in freshwater ecosystems.

To be noted that the species composition during the hydrobiological studies till now were highlighting peculiarities of mostly investigated species from food (as fish feed) perspective rather than from their theoretical value. That is a reason why the organismz amount (number or biomass) per the definite single territory or volume was in a forefront during the hydrobiological studies until the recent times. But now the main focus of the reserches are population number, their structure, the ratio of individuals etc. As a hidrobiological research object of the coutries fresh water ecosystems, the animals of three large groups of two differing from each other by their structure and life activities - semi-kingdoms (single-cellular and multicellular organizms) are most attractive. It includes the a single-celled, invertebrate and vertebrate animals. Via taking into account the animals natural development peculiarities we decided to start this review with the protozoa.

Protozoa semi-kingdom representatives are very widespread on the planet. Those are encountered in the seas and oceans, freshwater courses and land, and in the stage of cyst in atmosphere air, in animals - in the gaps between their organs and tissues. We intend to provide here a brief summary on the relatively detailed studied from species composition perspective group - free-living infusors (*Oligohymenophora*) and other groups.

SINGLE-CELLULAR ORGANISMS SEMI-KINGDOM (PROTOZOA)

Currently, more than 200 thousand species of single-celled organisms are known and 20 thousand species of those are having parasites lifestyle.

I. Amoebas and Infusoria

More than 80 years have elapsed since the establishment of the Institute of Zoology of the Academy of Azerbaijan in 1936. Over the years, a number of scientific areas have emerged, not previously represented only in Azerbaijan, but also at the level of the former USSR. One of the firsts was the development of domestic protozoology, ichthyology, ornithology and other directions of Zoology.

Since then, the Institute of Zoology has been replaced by several generations of researchers who have contributed to the study of the animal world of Azerbaijan. It should be noted, however, that almost all of our scientists' publications have always focused only on their facilities and general articles, which gave an idea of the overall level of biodiversity in the animal world, have not yet been published.

In this paper, we have tried to combine the current results of many years of research into the most successful areas of domestic zoology-protozoology, parasitology, hydrobiology, as well as herpetology, ornithology and others. Accordingly, each of these sections is written by experts in these groups.

At present, some 200 thousand are known. The simplest organisms, of which more than 20 thousand lead a parasitic way of life, including by causing and being carriers of dangerous diseases of animals and humans. No less species diversity and importance are free-living basic inlands in the seas, fresh waters and soils. Of the 200 thousand protozoa species today the 40,000 are the inhabitants of the seas and the oceans. Their role in the Earth's biological processes is enormous. Researches were carried out primarily on parasitic groups of the simplest - Coccidias. Different groups of animals-primarily rodents-were surveyed, of which 22 were found in only 125 species of *Coccidia*, 86 of which were described for the first time for science.

Data on free-living protozoans of Azerbaijan in the works of early authors is extremely small. The main source of literature is the chubar of S.Ya.Veisig (1940) "Microfauna of the Caucasus". In summing up the early authors, it is possible to say that before the modern period of research from the marine and freshwater waters of Azerbaijan, only 85 species of infusoria and 43 species of amoebas, both nude and sink, were known. The beginning of the current period of study free-living the simplest in Azerbaijan laid down the work of Agamaliyev on the Infuzoria of the Caspian Sea, which began in the mid-1960s. His multi-year studies have expanded our knowledge of the free-living infusorias of the Caspian - if only a few species were known according to the data of Grimm and

Veisig, then a monograph of Agamaliyev, "The infuzoria of the Caspian sea", published in 1983, lists 439 species, of which 1 genus and 20 species were described for the first time in science. In the 1980s, several more complex studies were conducted on the simplest pedobiontov of several taxonomic groups (flagellates, amoebas, infusoria). The simplest soils of citrus groves in Lankaran (R. Ibadov, 1983) and cultivated soils in Apsheron (N. Mirza-Zadeh, 1989) were investigated. It should be noted that these work are largely not fauna, but the environmental nature of the protozoa-pedobionts studies.

Since 1986, Azerbaijan has for the first time conducted a special study of soil shells amoebas. In the forest soils of northwest Azerbaijan, a total of 93 species of testacid were found, of which 39 species were first registered for the Caucasus fauna and 66 species for the fauna of Azerbaijan. In the 1970s, several complex hydrobiological studies were carried out (Aliyev, 1971, 1990) microbentos of freshwaters, where, along with other hydrobionts groups, free-living eat, of which about 20 species were first described for science.

However, the special studies of infusoria in water reservoirs of Azerbaijan were started by Alekperov in 1972. The taxonomic results of these years of work were published in 2005 in his monograph - "Atlas of free-living infusoria" which, on the basis of modern PAP methods impregnation silver, shows the illustrated descriptions of 230 infusoria species, of which 2 families, 8 genera and 90 species were described for the first time. A full summary of the results of 40-year studies on the species diversity of marine, freshwater and soil infusoria of Azerbaijan and other regions of the world, as well as environmental studies, including the use of some types of infusoria for biotesting at the cellular and population levels, were summarized in the second monograph - "Free-living infusorias of Azerbaijan" of Alekperov published in 2012. At present, our research on the species diversity of the free-living Infusoria of Azerbaijan is estimated at about 750 species, and for the first time, 3 families, 11 genera and 104 species were described for science. After more than 60 years break, at the beginning of the new millennium, studies of the freshwater sinks of the amoebas of Azerbaijan were resumed and the results showed the high diversity of the group. The fauna of the testacid freshwater of Azerbaijan is now represented by more than 120 species, of which two families, eight genera and 47 species were first described for science (N. Snegovaya, I. Alekperov, 2005).

Thus, the multiyear research of the Azerbaijani protozoologists, both in the field of parasitic protozoologii and in the study of the free-living simplest

have made a great and recognized contribution to the study of the biodiversity of the simplest, the best evidence of the 86 of new species in the Coccidia, as well as 5 families, 18 genera and 176 new types of free-living infusoria and shell amoebas.

Natural water basins of a free-living ciliates (*Ciliata*). As mentioned above, there is no water course where no infusoria representative (species) is found during the various seasons of the year or various temperature conditions any representatives not found (species) ciliates tutarı ahead. In freshwaters of Azerbaijan more than 600 infusoria species were recorded, of them 30 species are described for the science for the first time (A.Aliyev, 1987, 1990, 1991 and others). The following infusors are taken the key role in forming the biodiversity accordingly in various ecosystems: in stagnant watercourses, primarily lakes and ponds – the representatives of the genres *Coleps*, *Lacrymaria*, *Holophrya*, *Spirostomum*, *Metopus*, *Paramecium*, *Frontonia*, *Euplotes*, *Oxytricha*, in flowing watercourses – rivers springs the representatives of genres *Nassula*, *Zosterodasys*, *Euplotes* etc. are having the predominant development. *Paramecium caudatum*, *P.dragescoi*, *Frontonia leucas*, *F.acuminata*, *Spirostomum teres*, *S. minus*, *Metopus es*, *M. are frequently found in our fauna*.

Not only in water ecosystems, also in all inhabiting environment the infusors play key role in matter and energy cycle, in restoration of environments natural quality and forming of natural-biological indicators of it. There are many species with economic and epidemiological value among them - *Paramecium* *Frontonia* *Euplotes*, *Condilostoma*, *Metopus* and a number of other species.

MULTI- CELLULAR ORGANISMS SEMI-KINGDOM (METAZOA)

II. Invertebrates (*Invertebrata*). This group's species covers more than 98% of all animals. It is one of the main components of water-course hydrofauna and the representatives are formed in 20-30 groups. This figure varies depending on the type, features of watercourses as well as the water chemical composition. They play key role in energy flow in ecosystems, food chain, in forming of qualitative indicators of water and in its saprobic performance etc. .

Now let us go through some stand alone groups:

1. Sponges (*Porifera*) – no special research studies were conducted on this group, has been investigated not deeply. Three species (*Spongilla lacustris*, *S.carteri*, *Ephydatia fluviatilis*) were registered in watercourses (Derjavin, 1951; Gasimov, 1972).

Playing role in the energy exchange. These are active filtrators of water in the environment. Climate activists filtratorlarıdır water.

2. Coelenterates (*Coelenterata*) – no special research studies were conducted on this group as well. 3-4 species are known in our fauna. The majority of them were registered during the hydrobiological surveys. In stagnant waters *Hidra oligactis* və *H.vulgaris* are common inhabitants. *Microhydra sowerbii* is spread in Kura river and Jandar lake (Gasimov, 1972, 2002). Has a rol in food chain and biological treatment of the water.

3. Turbellaria – despite being very important component of mountain rivers' ecosystems the species composition of this group is studies very weak. In water courses (particularly in river mountains) five species (*Dugesia gonocephala*, *D.polychroa*, *Dendrocoelum lacteum*, *Mesostoma productum*, *Microstomum lineare*) and three subspecies (*Dugesia g.transcaucasica*, *D.g.bakurianica*, *D.g.praecaucasica*) were registered (Gasimov, 1972, 2002). It is spread on the stones and plants in the stony biotopes rich with water plants. The widespread species is *Dugesia gonocephala* (Animal world of Azerbaijan, 2002).

4. Hairybacks (*Gastrotricha*) – not deeply studied group of the watercourses microfauna. Only two species (*Polymerus nodicaudus* və *Lepidoderma aguamata*) are registered (Aliyev, 1971) in freshwaters of our country, particularly in the areas rich with plant in Jeyranbatan water reservoir. Mostly found in plant communities with sphagnum.

5. Nematods (*Nematoda*) – are free-living mesobentic organisms. No specific reserches were conducted on this group in the freshwaters of the country. Three species in total (*Tripula papillata*, *Rhabdolaimus aquaticus*, *Diplogaster rivalis*) were registered (Aliyev, 1971) during the reserch studies conducted at microbentos Jeyranbatan water reservoir. Also as per info of A.Gasimov (1972) two species (*Tripula papillata* and *Dorilamus stagnalis*) were found in Khalifachay river in the vicinity of Shusha city. So, four species of this group is known from our country. In 1970s a resercher so called Sakharova conducted special reserch studies in this direction in Mingechaur water reservoir, however we have no info in species composition.

6. Oligochaetes (*Oligochaeta*) – an important component of water ecosystems, plays role in fish food, forming of soil and biological treatment of the water. 90-109 species were registered in waters of Azerbaijan. (Gasimov, 1965, 1972, Animal world of Azerbaijan, 2002; Aliyev, 1971, 2010). The widespread species are from the genres of *Stylaria*, *Nais*, *Chaetogaster*, *Limnodrilus*, *Tubifex*

etc. Playing role in matter and energy cycle as well as the saprobic conditions determination in the environment.

7. Leeches (Hirudinea) – no special research studies were conducted on this group. 15 (± 2) species were registered in our water courses (Animal world of Azerbaijan, 2002). The most common species are *Haementeria costata* and *Hirudo medicinalis* and close to them species. Currently (2017) experiments are being conducted for reproduction of medical leeches (*H.m.orientalis*) in the lab conditions. The results were promising.

8. Rotatoria – is the multi-species group of the plankton - organisms living in the water columns. More than 289 species (300 species) have been registered in Azerbaijan (Gasimov, 1984). From species number perspective *Barchionus* (33 species), *Trichocerca* (27 species), *Lecane* (26 species) *Keratella* (10 species) and other genera are differing. *Rotatorias* have a role in ecosystem's food chain, matter and energy exchange.

9. Molluscs (Mollusca) – so far 63 species (in some sources 47 species) were registered in Azerbaijani waters. Most of these species belong to gastropods ($\approx 85\%$), the some (12-15%) to bivalves (Gasimov, 1972, Animal world of Azerbaijan, 2002). The widespread species are from the genera *Lymnaea* (25 species), *Costatella*, *Planorbis*, *Theodoxus*, *Melanopsis*, *Unio*, *Anodonta*, *Corbicula* etc. This group species are contributors to the ecosystem's matter and energy exchange, water **purification** and to reproduction of parasite worms. Many species are of economic value.

10. Briozoa – species composition has been poorly investigated and consist of only four species (Animal world of Azerbaijan, 2002). *Plumatella fungosa* və *P.punctata*, *P.emarginata* are frequently encountered in republic water bodies.

11. Anostraca – representatives are widespread in salty waters. 5 species (Animal world of Azerbaijan, 2002) are inhabiting in Azerbaijan. *A.salina* species of *Artemia* genus is the key species which is the permanent inhabitant of salty and hyperthick water bodies. *Branchinecta media* and *Branchinecta ferox*- species are also specific to salty lakes in Absheron. *Chirocephalus skorikowi* and *Ch.weisigi* are endemics of the Caucasus. This group representatives are having a role in biological purification of the environment, matter and energy exchange. *A.salina* cancer is considered as the most valuable organic food in aquaculture.

12. Notostraca - poorly investigated group with two species (*Triopis concoloriformis* and *T.c.transcaucasicus*) in water bodies. The latter is the endemic of the Caucasus (Gasimov, 2002). Molluscs are having a role in biological purification of the environment, matter and energy exchange.

13. Water fleas (Cladocera) – are the plankton species and represented by 95-126 species in country water bodies (Animal world of Azerbaijan, 2004). *Daphnia magna*, *D.longispina*, *Simocephalus vetulus*, *Chidorus sphaericus* are the predominant species. From the tropic species *Diaphanasoma sarsi*, *D.atkinsoni*, *D.carinata*, *Ceriodaphnia reticulata*, *C.coronata*, *Macrothrix spinosa*, *Chidorus barroisi* and others widespread in our water bodies. **Water fleas are developing in our water bodies massively and are the main main food for fish.**

14. Copepods (Copepoda) – are one of the significant components of planktonic community Planktonik and are represented by 60 species from 3 orders - *Calanipeda*, *Cyclopeoidea* and *Harpacticoida*. In terms of biodiversity the order *Cyclopoida* with its 43 species comes first and is being followed by orders *Calanipeda* (14 species) and *Harpacticoida* (6 species). The widespread species in our water bodies belong to genera *Calanipeda* (predominant species is *C.aqual-dulsis*), *Sinodiaptomus*, *Acanthocyclops* and *Cyclops*. *Sinodiaptomus sarsi* is widespread in water bodies of plains, *Hemidiaptomus monticola* (an endemic species of Azerbaijan) of mountains, şortəhər sulu göllərdə isə *Arctodiaptomus salinus* is widespread in medium salty lakes (Animal world of Azerbaijan, 2004). Copepods are contributors to the ecosystem's matter and energy exchange, water purification and to reproduction of some parasite worms.

15. Ostracodes - (Ostracoda) – 19 species are registered in Azerbaijani water bodies. The dominant species are from genera *Ilyocypris* (*I.biplicata*, *I.getica*, *I.gibba*), *Cypris* (*C.bispinosa*, *C.pubera*), *Cyclocypris* (*C.ovum*), *Limnocythera* (*L.inopinata*, *L.stationis*). Ostracodes are contributors to the ecosystem's matter and energy exchange (Animal world of Azerbaijan, 2004).

16. Mysids (Mysidacea) – 2 species (*Paramysis lacustris* and *Lymnomyxis benedeni*) exist in freshwater courses. It is one of the poorly investigated groups. Mysids are having role in energy and matter exchange of ecosystems. the weak. Water basin in matter and energy in the role.

17. Isopods - (Isopoda) – 3 species (*Asellus aquaticus*, *A.monticola*, *A.infirmis*) are registered in our water bodies (Animal world of Azerbaijan, 2004). Isopods are having role in biodiversity and food chain.. *A.aquaticus* is the aquaculture object.

18. Amphipods - (Amphipoda) – are represented by 32 species in our freshwater courses (Aliyev, 2000). Species of genera *Gammarus* and *Pontogammarus* are the predominant ones. The amphipods of freshwater courses are originated from the autochthonous species of the Caspian sea (*Gammarus matienus*, *G.komareki*, *Synurella*

apscheronica, *Pontogammarus sarsi*, *P.robustoides*, *Dikerogammarus haemobaphes*), endemics of the Caucasus (*Niphargus abricossovi*, *N.komareki araxensis*) and Azerbaijan (*Gammarus balkanicus talysahensis*, *Lyurella hyrcana*, *Synurella apscheronica* vø b.). Amphipods play a significant role in ecosystem, biodiversity, and food chain and are widely used in aquacultures.

19. Decapods - (Decapoda) – are represented by up to 10 species in water courses, rivers, lakes, water reservoirs (Gasimov, 1972, Animal world of Azerbaijan, 2004). The key species are (*Astacus leptodactylus*, *A.pylzowi*), shrimps (*Paleomon adspersus*, *P.elegans* and some forms), crumps (*Potamon magnum*, *P.albanicum*, *P.ibericum* etc.). *A.pylzowi* is an endemic species. The most of the species of high crustaceans resistered in azerbaijani waters are of economic importance.

20. Tardigrades - (Tardigrada) – 3 species were regsieterd in the middle of the 20th centry–*Macrobiotus hufelandi*, *Milnesium tardigradum* and *Macrobiotus* sp. (Animal world of Azerbaijan, 2004; Aliyev, 1971). Uncommon organizms physically tolerant to high radiation (570000 x-ray) and maximum dehydration (up to 99 %), and may stay in long time anabiosis. Currently world wide reserch extensive studies (endurance to genetic and deteriorating conditions) are being conducted on these group representatives.

21. Mayfly larvae (Ephemeroptera, larva) – 40 species (33 larvae species, 7 adult species) are registered in water courses (Animal world of Azerbaijan, 2004). The widespread specieas are: *Isonychia ignota*, *Ameletus inopinatus*, *Syphlonurus linnaeus*, *Ordella macrura*, *Caenis macrura*, *Palingenia fluginosa*, *Ephemera vulgata*, *Potomanthus eluteus*, *Cloeon dipterum* etc. Among the mayfly larvae *Ecdyonurus znojkoii*, *E.flater*, *E.ornatipennis*, *Cinugra caucasis*, *Iron zojkoi* are the endemic species for the Caucasus. The group representatives are significant parts of food chain and matter exchange.

22. Dragonflies larvae Odonata, larva) –60 species were registered in our water courses, of them only 41 species were found in the waters of Lenkoran natural province (Animal world of Azerbaijan, 2004). The widespread species of Republic's water bodies are – *Ischnura elegans*, *I.pumilio*, *Coenagrion concinnum*, *C.hastulatum*, *C.pulchellum*, *C.puella*, *C.scitulum*, *Anax imperator*, *Orthetrum brunneum* etc. Are valuble organizms and food for birds and reptiles. Are feeding on the trees during the mature/adult stage.

23. Stoneflies (Plecoptera, larva) – 32 species were registered (Animal world of Azerbaijan, 2004). Are inhabitants of pure water courses with lower temperature and rich O₂ conditions –

particularly in mountain rivers, stony biotopes. *Perla pallida*, *Isoperla caucasica*, *Chloroperla tripunctata*, *Ch.grammatica* etc. Are widespread in mountain rivers. More than 60% (about 20 species) of stoneflies registered in Azerbaijan are endemics of the Caucasus. This includes *Perla kiritshenkovi*, *P.caucasica*, *P.pallida*, *Isoperla caucasica*, *I.pulchra*, *Chloroperla tripunctata*, *Ch.teberdinica*, *Ch.grammatica*, *Leuctra minuta*, *L.simplex*, *Nemura brevipennis*, *Protonemura bitida*, *P.alti cola*, *P.vernalis*, *P.viridis*, *Capnia arensi*, *C.tyberculata*, *Esera caucasica* etc.. Stonflies have significant role in ecosystem's food chain and are indicators to determine the level of water contaminatin by organic substances.

24. Caddisfly larvae (Trichoptera, larva) - 45 species were registered in internal water bodies (Animal world of Azerbaijan, 2004), are inhabitants of mountain rivers and springs.

Are encountererd in stagnant and pure water bodies, mostly rheophilic species. The most common species are: *Ryacophila talyschica*, *Hydropsyche gracilis*, *H.ornatula*, *H.acuta*, *Ecnomus tenellus*, *Limnophilus affinis*, *L.znojkoii*, *L.fluvicornis* etc. *Psychomyia shelkovnikovi*, *Cyrnus trimaculatus* species are dominating in small rivers in the territory of the Gey-Gel Natioanal Park, when *Polycentropus flavomaculatus*, *Hydropsyche pellicidula* species are dominating by number in the rivers of the Greater Caucasus. 14 of the registered species (*Ryacophila talyschica*, *Rh.cupressorum*, *Glossosoma unquiculatum*, *G.capitatum*, *Hydropsyche acuta*, *H.gracilis*, *H.subguttata*, *Lithax incanus*, *Ernodes palpata*, *Lymnophilus flavicornis*, *L.znojkoii*, *Glyphotaelius selysi*, *Psiloptera perzovi*, *Micrasema bifoliatum*) in Azerbaijan territory are the endemics of the Caucasus. As an indicator of the clean waters this group also has a role in ecosystem's matter and energy exchange.

25. True bugs (Hemiptera). 870 species of 23 families are known in fauna of Azerbaijan (Animal world of Azerbaijan, 2004). 30 species (*Corixidae*, *Nepidae*, *Naucoridae*, *Aphelochiridae*, *Pleidae*, *Hydrometridae*, *Vellidae*, *Gerridae*) of 8 families (Gasimov,1972) were registered in the water courses. No special studies were conducted over the species inhabiting the water bodies. The up to date species number (30 species) of this group has been established based on the materials collected during the hidrobiological surveys. The most common species of our watercourses are: *Corixa punctata*, *C.dentipes*, *C.affinis*, *Notonecta glauca*, *N.lutea*, *N.viridis*, *Nepa cinerea*, *Ranatra linearis*, *Hydrometra stagnorum*, *Gerris Lacustris*, *G.thoracicus*, *Limnopus rufoscutellatus*, *Aquarius paludum*. The group representatives as one of the

links in food chain are contributing to matter and energy exchange.

26. Beetles (Coleoptera). 4000 species are known in Azerbaijan (Animal world of Azerbaijan, 2004), of them 124 are registered in larvae and mature stages in watercourses (Gasimov, 1972). All known species belong to 6 families (*Halipilidae*, *Ditiscidae*, *Girinidae*, *Hydrophilidae*, *Dryopidae*, *Chrysomelidae*) (Gasimov, 1972). Those are reaching their mass development in the parts rich with water plants in mostly stagnant waters. These species are frequently encountered in the lakes and ponds along the Kura river as well as in watercourses of the Lenkoran natural province. Su tutarlarımızda rast gəlmə intensivliyinə görə (*Halipilus ruficollis*, *H. fluviatilis*, *H. flavicollis*, *Noterus clavicornis*, *Laccophilus hyalinus*, *L. minutus*, *L. variegatus*, *Hydrovatus cuspidatus*, *Hydroporus planus*, *H. palustris*, *H. tessellatus*, *Cybister lateralis marginalis*, *C. tripunctatus*, *Gyrinus columbus*, *G. minutus*, *G. caspius*, *Hydrous piscus*, *Enochrus testaceus*, *Laccobius pallidissimus*, *Heteroserus flavidus* etc.) are the in leading position in terms of encountering intensity in the watercourses. Most of the larvae have predator type of life. Group representatives play role in productivity of watercourses, as well as in matter and energy exchange.

27. True flies (Diptera) – has got reach species composition in the watercourses' ecosystems and the most important families encountered during the hydrobiological surveys are: - biting midges, black flies and chironomid larvae.

biting midges larvae (Ceratopogonidae, larva) were studied comprehensively by Sh. Jafarov (1964). More than 15 genera of this family were investigated. The most valuable genus in terms of hydrobiological peculiarities is *Culicoides*. 62 species were determined (Animal world of Azerbaijan, 2004). Most of the representatives in larval stage in water habitat (wetlands) feed on detritus and decay, in mature stage suck the blood. These are the permanent inhabitants of the swamps/wetlands. The most common species are the following: *Culicoides nubiculosus*, *C. puncticollis*, *C. circumscriptus*, *C. salinaris*, *C. polycaris*, *C. picipennis*, *C. caspius* etc. The group representatives participants of ecosystem's matter and energy exchange and have an epidemiological importance.

Black flies, larvae (Simuliidae larva). 43 species have been determined in Azerbaijan. Key reproduction areas of them are in the Central Aran and Kura river valley. Feed on blood (sucks) in the stage of larvae, lead to sickness and mortality of cows, buffalos and other livestock. Key pests are kura black fly - *Simulium kurenze* and znoyka black fly - *Cnephia znoikoi* (Jafarov, 1960). The representatives of genera *Odagmia* (*Od. varegata*,

Od. caucasica, *Od. alasensis hiemalis*, *Od. monticola*) and *Obuchova* (*Ob. popovae*) predominate in the mountainous and lower zones of the Greater and Minor Caucasus, when in Talysh zone *Od. caucasica*, *Ob. popovae*, *Odagmia variegata* species are widespread (Animal world of Azerbaijan, 2004).

Nonbiting midges larvae (*Chironomidae larva*). This family has the highest species diversity among dipteras, 2000 species are known in globe, up to 150 species (basically 139 species) were identified in fresh waters of Azerbaijan, (Animal world of Azerbaijan, 2004) növu qeydə. Nonbiting midges larvae are the main components in the benthic community in the water bodies and have particular importance in the matter and energy exchange of the ecosystems.

The massive reproduction of the following species were recorded accordingly in the indicated locations: *Cryptochironomus defectus*, *Psectrocladius psilopterus*, *Cricotopus silvestris* – in downstream of Kura river, *Tanytarsus gregarius*, *Chironomus plumosus*, *Ch. salinus*, *Einfeldia pagana*, *Limnochironomus nervosus* – in Hajigabul lake *C. mancus*, *M. lobatifrons*, *Glyptotendipes gripekoveni*, *Ch. plumosus*, *Diamesia longiper* – Geoy-Geol, *Harnischia burganadzeae*, *P. coniectum*, *P. scalaenumi*, *Procladius ferrugineus* etc. – Jeyranbatan water reservoir (Animal world of Azerbaijan, 2004; Aliyev, 1971).

10 species of the known *Chironomid* larvae from the republic's water bodies (*Tanytarsus sevanicus*, *Harnischia burganadzeae*, *Cryptochironomus pankratovae*, *Eukiefferella quadridentata*, *E. popovae*, *Synorthcladius murvanidzei* və s.) are the endemics of the Caucasus (Animal world of Azerbaijan, 2004).

Microzoobentos of the rivers. 248 species of microbenthic organisms form 25 taxonomic groups have been revealed from the waters of Azerbaijan. 160 species of 22 taxonomic groups have been registered from the Kura river. Of them 21 species belong to molluscs, 20 – to odonates larvae, 16-oligochets and 15 to mayfly larvae.

95 macrobenthic organism species were determined from the Araz river and its branches. 79 species of them belong to water insects, and 19 to mollusks. The key position belongs to odonates' larvae (11 species), mayfly larvae (10 species), Phryganea larvae (8 species) and others.

134 species of microbenthic organisms form 18 taxonomic groups were identified in rivers of Nakhchivan AR (Nakhchivan river – 77, Eastern Arpachay – 68, Salarsuchay – 27, Jahrichay river – 21, Bichanakchay – 35, Gilanchay – 55, Ordubadchay – 54, Eylischay – 60). The sequence based on the encountering intensity of the revealed species

in the study watercourses is listed below: *Dero dorsalis*, *Ilyocypris getica*, *Dikerogammarus haemobaphes*, *Gammarus lacustris*, *Costatella acuta*, *Aplexa hypnorum*, *Agrion virgo*, *Coenagrion scitulum*, *Palingenia longiscauda*, *Ordella macrura*, *Micronecta pusilla*, *Hydrometra stagnorum*, *Hydropsyche ornatula*, *Leptocerus tineiformis*, *Culex pipiens*, *Tanutarsus gregarius*, *Chironomus thummi*, *Leptoconops caucasicus* etc.

The following macrobenthic organism were revealed in the rivers of the north-eastern zone of the Greater Caucasus (Gusarchay 49, Gudyalchay 44, Aghchay 50, Garachay 53, Velvelachay 29, Shabbranchay 28, Devachichay 34, Atachay 12, Gilgilchay 16). Molluscs, mayflies, and odonata larvae predominate in studied rivers.

The benthic fauna in the rivers of the north-western part of the Greater Caucasus (Alazan, Katekhchay, Kurmukchay, Eyrichay, Kishchay, Shinchay, Silbachay, Balakanchay), of the southern part (Turyanchay, Vendamchay, Geoychay, Girdimanchay, Demiraparanchay, Bumchay, Akhsuchay, Akhokhchay) have been revealed. 60 species from the north-western zone rivers, and 102 species of benthic organisms were determined from the southern slope rivers. The species number varies between 32-64 in the rivers of the area. The sequence on species encountering frequency is the following: *Nais communus*, *Branchiura soverbyi*, *Hydrobia longiscata*, *Ecnomus tenellus*, *Hydropsyche ornatula*, *Limnophilus flavicornis*, *Leptocerus tineiformis*, *Oecetis furva*, *Procladius choereus*, etc. The littoral species are dominant in the rivers.

The revealed species numbers are shown accordingly per the rivers in the southern regions of Azerbaijan: - Astarachay 56 species, Lenkaranchay 64, Girdanichay 48, Veravulchay 42, Boladichay 54, Gumbashichay 68, Vilashchay 56 species of organisms. Most of the determined species in the rivers belong to and fito- and litoreofil types. 48 benthic organism species were identified in the Absheron-Gobustan region rivers (Jeyran-kechmazchay, Pirsatchay, Sumgayitchay). The species split is the following: 42 in Pirsatchay, 36 – in Sumgayitchay, 14 – in Jeyrankechmezchay. Stony habitat is dominating in the rivers.

Kurekchay, Terterchay, Hachinchay, Gargarachay, Injachay rivers flowing in the Karabakh volcanic plateau of the Lesser Caucasus, located in the of 114 species of organisms were found. The split of the found species per rivers is the next: Terterchay - 86 species, 94 species - Khichinchay, 74 species - Gargarachay, 80 species - Kurakchay, 66 species – Injachay. These rivers fauna is based on nonbiting midges larvae, trichopters, mayfly larvae, molluscs.

III. VERTEBRATA

Although vertebrates are inferior to unicellular organisms and invertebrates in terms of their biodiversity they have a significant economic importance and role in the ecosystem. There are representatives of all of their groups in our fauna. Along with sprat and sturgeon fish that are important for fisheries, cyclostomata can also be found in our water resources.

Pisces. One species of cyclostomata and 109 species and subspecies of fish were recorded in the domestic water basins of Azerbaijan (Ibrahimov, Mustafayev, 2015). 9 of the species [*Salmo ischchan*, *Carassius auratus gibelio*, *Pseudorasbora parva*, *Hemiculter leucisculus*, *Gambusia affinis*, *Gasterosteus aculeatus*, *Anguilla anguilla*, *Liza aurata* and *Liza saliens*] were brought either specifically or accidentally. At present, the internal water basins of Azerbaijan have Caspiomyzon wagneri and 67 species and subspecies of fish. 59 of them are of freshwater and 8 are of saltwater origin.

Petromyzontidae family. One representative of the Petromyzontidae family – *Caspiomyzon wagneri* lives in the waters of Azerbaijan. *Caspiomyzon wagneri* live in the sea, while mature individuals enter rivers to spawn. Biological indicators of the Kura population of the *Caspiomyzon wagneri* are much higher than those of the Lankaran population. Spawning of the *Caspiomyzon wagneri* occurs in May-June (Mustafayev, 2013).

Acipenseridae family. There are 4 species belonging to 2 genera of the Acipenseridae family in the internal water basins of Azerbaijan (lower Kura). One of them – *Acipenser nudiiventris* has been included in the Red List of Azerbaijan since 2013, while industrial hunting of others (*Huso huso*, *Acipenser persicus* and *Acipenser stellatus*) was suspended in 2010.

Salmonidae family. There are 2 species belonging to 1 genus of the Salmonidae family in the internal water basins of Azerbaijan. One species (*Salmo ischchan*) is an endemic fish of Lake Goycha. It was released to the mountain lakes of the Kalbajar region (Maral-gol, Boyuk Alagol and Kichik Alagol) in order to acclimatize in 1977-1980. Two years after their release to the lakes, their masses reached 500-700 g. Sex glands of female individuals hunted from Maral-gol in December was at the 4th stage of puberty. The fact that younglings were caught together with large fishes in subsequent years gave reason to believe that this species were adapted to new ecological conditions (Guliyev, 2006). The reserves of each of the 2 subspecies (*Salmo trutta fario* and *S. trutta caspius*) of the other species dropped sharply and they were included in the Red List of Azerbaijan.

Esocidae family. This family is represented by one species (*Esox lucius*) in Azerbaijan's fauna. This species is found in the lower streams of the great rivers and canals, flowing through the territory of Azerbaijan and directly into the Caspian Sea, as well as in the lakes around Kura, Varvara reservoir, Davachi port and Kichik Gizilagaj Gulf.

Cyprinidae family. Until recent years there have been information maintaining that 35 species and subspecies belonging to 22 genera of the *Cyprinidae* family were spread in the domestic water basins of Azerbaijan (Animal World of Azerbaijan, 2004).

Pseudorasbora parva and *Hemiculter leuciscus* that belong to this family were recorded our fauna in 2012 and 2013 respectively (Mustafayev, Ibrahimov, 2012; Mustafayev, 2013). Those species have been brought coincidentally during the acclimatization of herbivore fish in the waters of Azerbaijan and are very widespread today. Both species (especially *Pseudorasbora parva*) have a negative impact on fish resources, since they both compete for food with and eat caviar of the representatives of local fauna.

There are 37 species belonging to 24 genera of the *Cyprinidae* family in the internal water basins of Azerbaijan today. 5 of the species and subspecies (*Rutilus atropatenus*, *Luciobarbus capito*, *Pseudorasbora parva*, *L.brachycephalus caspius*, *Pelecus cultratus*, *Abramis sapa bergi*) were included in Azerbaijan's Red List. There are representatives of this family in almost all of the domestic water basins of Azerbaijan.

Balitoridae family. 3 species [*Barbatula angorae*, *B.merga* and *B.brandti*] and one subspecies [*B.a.lenkoranensis*] of 1 genus of this family are spread in the internal water basins. The *Balitoridae* are typically freshwater fish and live in rivers with rocky-gravel bottom and relatively fast flow.

Cobitidae family. There are representatives of 3 species (*Cobitis taenia satunini*, *Sabanejewia aurata* and *S.caspia*) of 2 genera of this family in the internal water basins. The *Cobitidae* that are typical freshwater species are spread in rivers, lakes around Kura, and reservoirs.

Siluridae family. One species (*Silurus glanis*) of this family inhabits the internal water basins of Azerbaijan. Both semi-anadromous populations and sedentary populations always living in freshwater can be found in Azerbaijan's water courses.

Gasterosteidae family. Representatives of 2 species (*Gasterosteus aculeatus* and *Pungitius platygaster*) of this family live in the Caspian Sea. In order to reproduce they also descend into rivers flowing from the territory of Azerbaijan and into the sea.

Syngnathidae family. This family is represented by one subspecies (*Syngnathus nigrolineatus*

caspius) in the Caspian Sea. *Syngnathus nigrolineatus caspius* is sea fish which approach estuary during the reproduction period. It can live in freshwater for a long time. These fishes are found in rivers flowing into the Caspian Sea, canal mouths, at the Davachi port and in the Kichik Gizilagaj Gulf.

Poeciliidae family. One species (*Gambusia affinis*) of this family was acclimatized in the water basins of Azerbaijan to fight malaria in the 1930s. It is one of the most widespread fishes in the country's inland water basins. It is spread in most water basins (rivers, lakes, water reservoirs, ponds, canals) that have a weak stream, vast plant development and those that became marshy areas.

Mugilidae family. Today young individuals of one of the species (*Liza aurata*) belonging to this family, which was brought into the Caspian Sea to get acclimatized in 1930s, can be found in on the mouths of rivers flowing into the Caspian Sea, at the Davachi port and in the Kichik Gizilagaj Gulf.

Atherinidae family. One species of this family (*Atherina boyeri caspia*) is widely spread in the Caspian Sea. Large quantities can be found in areas close to the coast. They inhabit mouths of the rivers that flow into the Caspian Sea, canal mouths, Davachi port and the Kichik Gizilagaj Gulf.

Percidae family. 2 species (*Perca fluviatilis* and *Sander lucioperca*) of 2 genera of this family can be found in the internal water basins of Azerbaijan. *Perca fluviatilis* is represented only by freshwater populations, while *Sander lucioperca* has both semi-anadromous and freshwater populations in Azerbaijan's the internal water basins.

Gobiidae family. Representatives of 6 species (*Knipowitschia caucasicus*, *Neogobius melanostomus*, *Neogobius platyrostris constructor*, *Neogobius fluviatilis pallasii*, *Neogobius kessleri gorlap*, *Proterorhinus marmoratus*) belonging to 3 genera of this family live in the Caspian Sea. Only one of them (*Neogobius platyrostris constructor*) is freshwater, remaining are saltwater fish. However, they also have populations living in freshwaters.

Part of the cyclostomata and fish spread in the domestic water basins of Azerbaijan are spread in the majority of the water basins, another part lives only in reservoirs in certain areas, while the third group inhabit mouths of the rivers that flow into the Caspian Sea. Therefore, in order to provide detailed information about the fish that live in watercourses isolated with different geographical barriers, Azerbaijan's inland water basins can be conditionally divided into 5 regions (Lower Kura, Middle Kura, Nakhchivan, Northeastern and Southeastern water basins). 56 of all species and subspecies are spread in the Lower Kura, 39 in the Middle Kura, 28 in Nakhchivan, 35 in Northeastern and 47 in Southeastern regions.

Biodiversity of fish parasites. Although fish parasites were first studied in Azerbaijan in 1931-1932 in the vicinity of Sara Island in the Caspian Sea (Dogel and Bihovskii, 1939), ichtioparasitological studies in inland waters only started in 1949 (Nechaeva, 1964), and then successfully continued by a number of researchers (Mikailov, 1958, 1975; Pashaev, 1970; Abdullaeva, 1971; Ibrahimov, 1977, 2012; N.Aghaeva, 1982; Gazieva, 1984; Mehdieva, 1993; B.Agayeva, 2003; Guliev, 2006; Suleymanova, 2007; Badalova, 2011; Abdullayeva, 2013, and others). Here, we will try to list the number of species and characteristic species of parasites in Azerbaijan's fauna recorded in the fishes living in the country's water reservoirs.

Mastigophora division, *Kinetoplastomonada* class – 11 species: *Trypanosoma carassii*, *T. luciopercae*, *T. markewitschi*, *T. percae*, *T. schulmani*, *Cryptobia borelli*, *C. branchialis*, *C. cyprini*, *C. guerneorum*, *C. khaibulaewi*, *Costia necatrix*.

Sporozoa division, *Coccidiomorpha* class – 4 species: *Goussia alburni*, *Eimeria carpelli*, *E. percae*, *E. rutili*.

Microsporidia division, *Microsporidea* class – 5 species: *Glugea anomala*, *G. luciopercae*, *G. schulmani*, *Pleistophora siluri*, *P. sulci*.

Myxozoa division, *Myxosporidia* class – 59 species: *Myxidium lieberkuehni*, *M. macrocapsulare*, *M. pfeifferi*, *M. rhodei*, *Zschokkella nova*, *Z. sturionis*, *Sinuolinea sakinchanumae*, *Sphaerospora carassii*, *S. elegans*, *Chloromyxum cristatum*, *Ch. fluviatile*, *Ch. legeri*, *Ch. varicorhini*, *Myxobilatus gasterostei*, *M. medius*, *M. varicorhini*, *Myxosoma ranchiale*, *M. circulus*, *M. dujardini*, *Myxobolus albovae*, *M. alburni*, *M. alievi*, *M. azerbaijanicus* and others (total of 35 species in this genus), *Henneguya chaibulaevi*, *H. lobosa*, *Thelohanellus isgurni*, *Th. pyriformis*.

Ciliophora division, *Cyrtostomata* class – 2 species: *Chilodonella hexasticha*, *Ch. piscicola*.

Hymenostomata class – 2 species: *Tetrahymena pyriformis*, *Ichthyophthirius multifiliis*.

Suctorina class – 28 species: *Capriniana piscium*, *Scyphidia caligula*, *Epistilis lwoffii*, *Apiosoma amobae*, *A. baueri* and others (a total of 8 species), *Apisoma mikailovi*, *A. piscicolum*, *Trichodina acuta* and others (a total of 10 species) *Paratrachodina alburni*, *Tripartiella bulbosa*, *T. copiosa*, *Trichodina epizootica*, *T. subtilis*.

Coelenterata division, *Polypodinea* class – 1 species: *Polypodium hydriforme*.

Plathelminthes division, *Monogenea* class – 69 species: *Dactylogyrus chramulii*, *D. lenkorani*, *D. zandti* and others (total of 43 species), *Siluridiscoides magnus*, *S. siluri*, *S. vistulensis*, *Ancyrocephalus paradoxus*, *A. percae*, *Tetraonchus monenteron*,

Gyrodactylus arcuatus, *G. schulmani* and others (total of 12 species), *Paradiplozoon pavlovskii* and others (total of 6 species) *Eudiplozoon nipponicum*, *Diplozoon paradoxum*.

Cestoda class – 18 species: *Caryophyllaeus fimbriceps*, *C. laticeps*, *Biacetabulum appendiculatum*, *Caryophyllaeides fennica*, *Bothriocephalus acheilognathi*, *Ligula colymbi*, *L. intestinalis*, *Digramma interrupta*, *Schistocephalus pungitii*, *Bothrimonus fallax*, *Proteocephalus filicollis*, *P. gobiorum*, *P. ocellata*, *P. osculatus*, *Siluritaenia siluri*, *Gryporhynchus pusillus*, *Neogryporhynchus chaeilancristrotus*, *Paradilepis scolecina*,

Aspidogastrea class – 1 species: *Aspidogaster limacoides*.

Trematoda class – 81 species: *Bucephalus polymorphus*, *Rhipidocotyle companula*, *Rh. kovalae*, *Sanguinicola armata* and others (total of 4 species), *Bunocotyle cingulate*, *Monovitella cyclointestina*, *Saccocoelium obesum*, *S. tensum*, *Dicrogaster contracta*, *Asymphylogaster abdurachmanovi* and others (total of 5 species), *Parasymphylogaster markewitschi*, *P. parasquamosa*, *Palaeorchis incognitus*, *Crepidostomum farionis*, *Bunodera luciopercae*, *Phyllodistomum angulatum* and others (total of 5 species), *Skrjabinopsolus semiarmatus*, *Azygia lucii*, *Orientocreadium siluri*, *Allocreadium baueri* and others (total of 7 species), *Acanthocreadium araxicum*, *A. talishensis*, *Nicolla skrjabini*, *Sphaerostomum bramae*, *S. globioporium*, *Pseudosphaerostomum caudotestis*, *Pronoprymna ventricosa*, *Echinochasmus perfoliatus*, *Diplostomum chromatophorum* and others (total of 13 species), *Tylodelphys clavata*, *T. podicipina*, *Bolboforus confusus*, *Hysteromorpha triloba*, *Conodiplostomum perlatum*, *Ornithodiplostomum scardinii*, *Posthodiplostomum brevicaudatum*, *P. cuticola*, *Apharhyngostrigea cornu*, *Ichthyocotylurus erraticus*, *I. pileatus*, *I. variegatus*, *Holostephanus ubinini*, *Mesostephanus appendiculatus*, *Paracoenogonimus ovatus*, *Clinostomum omplanatum*, *Opisthorchis felinus*, *Ascocotyle coleostoma*, *Pygidioopsis genata*, *Metagonimus yakogowai*, *Cryptocotyle concave*, *Apophallus donicus*, *A. muehlingi*.

Nemathelminthes division, *Nematoda* class – 7 species: *Capillaria tomentosa*, *Thominx tuberculata*, *Cystoopsis acipenseris*, *Eustrongylides excisus*, *Rhabdochona denudata*, *Rh. fortunatowi*, *Rh. gnedini*, *Capillariospirura ovotrichuria*, *Cyclozone acipenserina*, *Desmidocercella numidica*, *Camallanus lacustris*, *C. truncatus*, *Molnaria intestinalis*, *M. leucisci*, *Agrachanus scardinii*, *Skrjabinianus tincae*, *Philometra ovate*, *Ph. rischta*, *Cucullanus sphaerocephalus*, *Spiroxis contortus*, *Anisakis schupakovi*, *Porrocoecum reticulatum*, *Raphidascaris acus*, *Contracaecum bidentatum* and others (a total of 3 species in this genus)

Acanthocephales division, *Acanthocephala* class – 27 species: *Neoechinorhynchus rutilis*, *Quadrigrus cholodkowskyi*, *Corynosoma capsicum*, *Leptorhynchoides plagicephalus*, *Acanthocephalus anguillae*, *A. lucii*, *Pomphorhynchus laevis*.

Annelida division, *Hirudinea* class – 4 species: *Piscicola fasciata*, *P. geometra*, *Hemiclepsis marginata*, *Limnotrachelobdella turkestanica*.

Mollusca division, *Bivalvia* class – 1 species: *Anodonta cyrea*.

Arthropoda division, *Crustacea* class – 12 species: *Lamproglana compacta*, *L. pulchella*, *Ergasilus briani*, *E. sieboldi*, *Thersitina gasterostei*, *Lernaea elegans*, *L. esocina*, *Caligus lacustris*, *Achtheres percarum*, *Pseudotracheliastes stellatus*, *Argulus coregoni*, *A. foliaceus*.

Overall, as a result of studies conducted by various experts, 332 species of parasites belonging to different classification groups of animals have been recorded in fish species living in Azerbaijan's inland water basins. 202 of those species (with the exception of *Cryptobia branchialis* and *Costia necatrix*) have a complex development cycle and use one or two intermediate hosts during such cycle. 52 species of parasites (including 37 species of trematodes, 14 species of nematodes and 1 species of thorny-headed worms) use the fish only as intermediate hosts and complete their development in fish eating birds and mammals.

77 species of parasites found in fishes of Azerbaijan's inland water basins cause serious diseases in fish, while representatives of 6 species (*Opisthorchis felinus*, *Metagonimus yokogawai*, *Apophallus muehlingi*, *A. donicus*, *Echinochasmus perfoliatus*, *Clinostomum complanatum*) cause disease when falling into people's digestive system. Cercaria of 36 species of trematodes, which use fish as the second intermediate host, attack the person entering the water, form small ulcers in the skin causing non-specific cercariosis.

Amfibia and Reptilia. Species of creatures living in the water basins vary depending on their depth, capacity, salinity, temperature regime and altitude. Amphibians and some species of Reptiles are important components of the aquatic ecosystem and mainly prefer freshwater basins. These freshwater basins are in flowing and stagnant state and are divided into several ecosystems, such as lentic, lotic and marshy areas [Mammadov, 2005].

11 species of Amphibians and 56 species of Reptiles have been recorded in our country. At present, 11 species of Amphibians and 4 species of Reptiles have been recorded in the inland water basins of Azerbaijan. These species are unevenly distributed in the freshwater ecosystems [Alakbarov, 1978; Ganiyev, 2012].

Lentic ecosystems (stagnant waters). There are mainly 2 species of Amphibians and 2 species of Reptile in stagnant water basins. One of them, *Hyla orientalis*, is found in permanent and temporary stagnant waters of the northern and western regions of Azerbaijan. During reproduction, the females spawn in 20-70 cm deep ponds. *Hyla savi-gnyi* on the other hand, found mostly in the permanent and temporary stagnant waters of the southern and south-western regions.

Emys orbicularis and *Mauremys caspica* that belong to Reptiles live in Lakes and other stagnant water basins in the plains and foothills and on their coasts. Both the *Emys orbicularis* and *Mauremys caspica* are recorded in the part of Davachi port which is rich in water plants [Ahmedov et al. 2015].

Lotic ecosystems (flowing waters). 3 species of amphibians live in such ecosystems. One of them, *Rana macrocnemis*, can be found in cold mountain rivers such as Shamkirchay, Gudialchay, Katekhchay as well as waterfalls and springs like Takdam and Laza. However, they prefer transparent and permanent water basins such as Goygol for reproduction. The spawning takes place at a depth of 30-40 cm in the water basins, in layers with the most favorable condition for the development of caviar.

Pelodytes caucasicus also lives in flowing waters like *Rana macrocnemis*. This species is found only in the north-western part of Azerbaijan, on the shores of Katekhchay, which flows through the dense part of the forest. They use springs and rivers with different depths (from 2-3 cm to 30-40 cm) to spawn.

Rivers passing through dense forest areas in the north-western region of Azerbaijan, for example, small ponds formed in the weak flowing part of Katekhchay and Silban rivers also have *Bufo verrucosissimus*. In order to spawn this species use temporary ponds with a depth of 25-30 cm, located on the edge of the river.

In addition to those listed, there are amphibians and reptiles linked to both stagnant and flowing water basins. *Triturus karelinii*, *Bufo variabilis* and *Pelophylax ridibundus* live in both weak flow and stagnant waters. Among them *Triturus karelinii* inhabit permanent stagnant waters on forest edges and in open areas of the forest and are not recorded in temporary water basins. Along with small basins, they can also be found in deep lakes of artificial origin. Chanlibel Lake and Gulustan Lake on the territory of Guba district, as well as other unnamed artificial lakes in Lankaran district are examples of this. During the reproduction period they attach their eggs to the bottom of water plants located at a depth of 10-25 cm, such as *Sparganium* spp. and *Mentha aquatica*.

Bufotes variabilis and *Pelophylax ridibundus* live in both permanent and temporary water reservoirs formed from rainwater within the territory of the country. *Bufotes variabilis* spawns on water plants, and sometimes on the bottom of the water in the parts of the basins not deeper than 50 cm and well-warmed by sun rays. *Pelophylax ridibundus* on the other hand spawns on water plants like *Potamogeton* spp. and *Sparganium* spp. in water basins with a depth of 30 cm to 1 m.

Pelobates Syriacus live on the sandy shores of the Caspian Sea, as well as on the shores of the flowing and stagnant water basins of Nakhchivan AR [Ganiyev, 2004]. Their reproduction takes place both in natural and artificial water basins, and they do not use temporary and cold water basins. The female members of this species lay caviar in plant rich water basins 5-15 cm deep.

Lissotriton vulgaris and *Bufo eichwaldi* are characteristic only for the flowing and stagnant water basins of the Lankaran natural province. Small dumps up to 40 cm are characteristic for tadpoles of *Bufo eichwaldi* [Kidov et al. 2009].

Natrix natrix and *Natrix tessellata* species that belong to Reptiles live both in flowing and stagnant water basins in plains, mountainous and foothill areas in Azerbaijan [Bunyatova, 2012]. *Natrix tessellata* can be found in Mazimchay in the north-western part of Azerbaijan, Shabbranchay in the northeastern part, in Astarachay in the southern part, in Ghizilagaj and Aggol south-eastern part, as well as in flowing and stagnant waters like Yukhari Shirvan channel and Bash Mil-Qarabagh Collector.

In addition to those listed above, there is a chance to find 1 more species of Reptiles in the water basins in the northwestern part of Azerbaijan. *Natrix megalcephala* was first recorded by Orlov et al. in Oguz district [Orlov et al., 1992]. Compared to other water snakes, it is stated that this species may be found in fast-flowing mountain rivers. However, this species has not been recorded by us.

Marshy areas. No stenobiont species are recorded for these areas. Because along with flowing and stagnant water basins *Pelophylax ridibundus*, *Bufotes variabilis* and *Pelobates Syriacus*, as well as *Emys orbicularis*, *Mauremys caspica*, *Natrix natrix* and *Natrix tessellata* of the Reptiles also live in this ecosystem. *Emys orbicularis* is widespread in the marshy areas of the north-eastern and southern-western regions of Azerbaijan.

It should be noted that 6 of 11 species of Amphibians found in domestic water basins of Azerbaijan (*Lissotriton vulgaris*, *Triturus karelinii*, *Pelobates syriacus*, *Pelodytes caucasicus*, *Bufo verrucosissimus*, *Bufo eichwaldi*) were included in the 2nd edition of Azerbaijan's Red List. 10 species of Amphibians and 4 species of Reptiles were also

included in the Red List of the International Union for Conservation of Nature in accordance with different categories and criteria.

Aves. There are 402 species in the fauna of Azerbaijan. The vast majority of them (about 305 species) are associated with the land - forests, woods, shrubs and meadows. A small part of them (≈ 97 species) is adapted to the water lifestyle. The main natural habitats of water and coastal birds in the modern time are Mahmudchala, Goychala, Hajigabul, Sharbat gorge, Boz gorge, Sarisu lake system, Mingachevir (65000 ha) and Varvara (15000 ha) reservoirs, ponds of fishery plants (about 3, 5 ha) in the Kura-Araxes lowland, Kichik Gizilagaj Gulf, Caspian and Aghgush water meadows in the Lankaran lowland.

Water and coastal birds settle down in various habitat biotopes like deep water areas, shallow water areas, reed, giant reed, sedge and reedmace jungles, open marshes and ravines, tamarisk jungles etc. Each of these biotopes has a unique ornithological complex. In total, 169 species of birds were recorded in these biotopes. Of those, 97 species are of water and coastal ecological group, and 73 species belong to open land and forest-bush ecological groups. These species are distributed unevenly on biotopes.

Deep water areas. It is mainly located in the central part of all the inland water basins. The depth of the water (excluding Mingachevir reservoir) varies between 0.6-0.7 m. These areas are big gathering places for the *Podicipediformes*, *Pelecaniform*, *Anseriformes* and *Fulica atra*. Their food facilities (zoobenthos, zooplankton and phytoplankton) are rich. 4 species of the *Podicipediformes* (*Podiceps ruficollis*, *P. cristatus*, *P. nigricollis*, *P. auritus*), 4 species of the *Pelecaniformes* (*Pelecanus onocrotalus*, *Pelecanus crispus*, *Phalacrocorax carbo*, *Phalacrocorax pygmaeus*), 31 species of the *Anseriformes* (*Rufibrenta ruficollis*, *Anser anser*, *A. albifrons*, *A. erythropus*, *Cygnus olor*, *C. cygnus*, *C. bewickii*, *Chen caerulescens*, *Tadorna ferrugine*, *T. tadorna*, *Anas platyrhynchos*, *A. crecca*, *A. strepera*, *A. penelope*, *A. acuta*, *A. querquedula*, *A. clypeata*, *A. angustirostris*, *Netta rufina*, *Aythya ferina*, *A. nyroca*, *A. fuligula*, *A. marila*, *Bucephala clangula*, *Clangula hyemalis*, *Melanitta nigra*, *M. fusca*, *Oxyura leucocephala*, *Mergus albellus*, *M. serrator*, *M. merganser*), only one species of *Gruiformes* (*Fulica atra*) and 1 species of the *Lariidae* (*Larus ichthyæetus*) settle in this biotope in winter and during migration. Three species of predatory birds (*Haliaeetus albicilla*, *Pandion haliaetus*, *Falco peregrinus*) can be found on the flight while searching for food.

Shallow water areas. Such areas are mainly seen in the natural water reservoirs (Aggol, Sarisu,

Sharbat gorge, Mahmudchala, Goychala, Aghzibirchala). Maximum water depth is 40-50 cm. Shallow water areas are rich in water invertebrates, fish younglings and amphibians, which are food for birds.

These areas are massive feeding sites for *Anseriformes*, *Ciconiiformes*, *Charadriiformes*. 9 species of the *Ciconiiformes* (*Ardeola ralloides*, *Bubulcus ibis*, *Egretta alba*, *E.garzetta*, *Nycticorax nycticorax*, *Ardea cinerea*, *A.purpurea*, *Platalea leucorodia*, *Plegadis falcinellus*) can be found in this biotope during the nesting period and when searching for food, as well as 1 species of the *Phoenicopteriformes* (*Phoenicopus roseus*) during the wintering period, 15 species of *Anseriformes* (*Anser anser*, *A.albifrons*, *A.erythropus*, *Rufibrenta ruficollis*, *Tadorna ferruginea*, *T.tadorna*, *Anas platyrhynchos*, *A.crecca*, *A.penelope*, *A.strepera*, *A.acuta*, *A.angustirostris*, *Netta rufina*, *Aythya ferin*, *A.nyroca*) during the wintering period and migration, 25 species of the *Charadriiformes* (*Vanellus vanellus*, *Vanellolochettusia leucura*, *Himantopus himantopus*, *Recurvirostra avosetta*, *Haematopus ostralegus*, *Tringa ochropus*, *T.glareola*, *Limosa limosa*, *T.totanus*, *T.erythropus*, *T.stagnatilis*, *Actitis hypoleucos*, *Xenus cinereus*, *Philomachus lobatus*, *Philomachus pugnax*, *Calidris minuta*, *C.temmincki*, *C.ferruginea*, *C.alpina*, *Limnocyptes minimus*, *Scolopax rusticola*, *Numenius arquata*, *Limosa limosa*, *Glareola pratincola*, *G.nordmanni*) when searching for food and during migration on the flight, and 2 species (*Limicola falcinellus*, *Gallinago gallinago*) during the wintering period, 2 species of the *Laridae* (*Larus argentatus*, *L.ridibundus*) during the wintering period, 2 species (*L.genei*, *L.minutus*) during migration, and 6 species (*L.melanocephalus*, *Chlidonias hybrida*, *Chlidonias leucopterus*, *C.niger*, *Gelochlidon nilotica*, *Sterna albifrons*) during the nesting period.

Reed, giant reed, sedge and reedmace jungles. They are located in various parts of the inland water basins. A large number of water and coastal birds such as the *Podicipediformes*, *Ciconiiformes*, *Anseriformes* and *Rallidae* can be seen here throughout the year. Large numbers of 4 species of the *Podicipediformes* (*Podiceps ruficollis*, *P.cristatus*, *P.nigricollis*, *P.auritus*), 1 species of the *Pelecaniformes* (*Phalacrocorax pygmaeus*) can be seen in this biotope, as well as 10 species of the *Ciconiiformes* (*Ardea cinerea*, *A.purpurea*, *Ardeola ralloides*, *Bubulcus ibis*, *Egretta alba*, *E.garzetta*, *Ixobrychus minutus*, *Nycticorax nycticorax*, *Platalea leucorodia*, *Plegadis falcinellus*) during the nesting period, and 1 species (*Botaurus stellaris*) during wintering and migration, 5 species of the *Anseriformes* (*Anser anser*, *Anas platyrhynchos*,

A.angustirostris, *Netta rufina*, *Aythya nyroca*) during the nesting period, 1 species of the *Rallidae* (*Crex crex*) during the wintering period, and 6 species (*Rallus aquaticus*, *Porzana porzana*, *P.pusilla*, *Gallinula chloropus*, *Porphyrio porphyrio*, *Fulica atra*) throughout the year, 35 species of birds that belong to forest-bush and open land ecological groups (*Circus pygargus*, *C.cyaneus*, *C.aeruginosus*, *Milvus migrans*, *Accipiter nisus*, *Cuculus canorus*, *Alcedo atthis*, *Merops apiaster*, *M.superciliosus*, *Coracias garrulus*, *Riparia riparia*, *Hirundo rustica*, *Delichon urbica*, *Pica pica*, *Corvus cornix*, *C.frugilegus*, *C.monedula*, *Parus caeruleus*, *P.major*, *Panurus biarmicus*, *Troglodytes troglodytes*, *Muscicapa striata*, *Turdus merula*, *T.pilaris*, *Acrocephalus arundinaceus*, *Acrocephalus schoenobaenus*, *Lanius minor*, *Lanius cristatus*, *Sturnus vulgaris*, *Pastor roseus*, *Emberiza schoeniclus*, *Fringilla coelebs*, *Carduelis carduelis*, *Passer montanus*, *Passer domesticus*).

Open marshes and saline areas. These biotopes are characteristic nesting places for such *Charadriiformes* as *Himantopus himantopus*, *Recurvirostra avosetta*, *Glareola pratincola*, *Chlidonias niger*, *C.leucopterus*, *C.hybrida*. 4 species of the *Ciconiiformes* (*Ardea cinerea*, *Bubulcus ibis*, *Egretta garzetta*, *E.alba*) can be found in these biotopes during migration, as well as 11 species of the *Anseriformes* (*Anser anser*, *A.albifrons*, *A.erythropus*, *Rufibrenta ruficollis*, *Anas platyrhynchos*, *A.crecca*, *A.penelope*, *A.strepera*, *A.acuta*, *A.angustirostris*, *A.querquedula*) during the wintering period, 2 species of the *Gruiformes* (*Grus grus*, *Antropoides virgo*) during migration, 6 species of the *Charadriiformes* (*Charadrius dubius*, *Vanellolochettusia leucura*, *Himantopus himantopus*, *Recurvirostra avosetta*, *Actitis hypoleucos*, *Glareola pratincola*) during the nesting period, 7 species (*Vanellus vanellus*, *Haematopus ostralegus*, *Tringa ochropus*, *T.glareola*, *T.nebularia*, *T.totanus*, *Gallinago media*) during the winter period and migration, and 8 species (*Tringa erythropus*, *Xenus cinereus*, *Philomachus pugnax*, *Calidris ferruginea*, *C.alpina*, *Numenius arquata*, *Limosa limosa*, *Glareola nordmanni*) throughout the year, 1 species of the *Laridae* (*Larus ridibundus*) during migration, and *Sterna albifrons* and *S.hirundo* during the nesting period.

Tamarisk jungles They are located as separate strips on the shores of the inland water basins. Tamarisk jungles have unique bird complexes in separate water basins. The main part of such complexes consists of the *Passeriformes*. 26 species of birds have been recorded in this biotope. Thoses include 6 species of predatory birds (*Aguila rapax*, *Circus cyaneus*, *C.pygargus*, *Buteo lagopus*, *Falco tinnunculus*, *F.subbuteo*), 2 species of the *Galli-*

formes (*Coturnix coturnix*, *Francolinus franco-linus*), 1 species of the *Charadriiformes* (*Scolopax rusticola*), 3 species of the *Columbiformes* (*Columba livia*, *C.palumbus*, *Streptopelia turtur*), 3 species of the *Strigiformes* (*Otus scops*, *Athene noctua*, *Asio flammeus*), 2 species of the *Coraciiformes* (*Coracias garrulus*, *Upupa epops*), 9 species of the *Passeriformes* (*Acrocephalus arundinaceus*, *Acrocephalus schoenobaenus*, *Lanius minor*, *L.cristatus*, *Pastor roseus*, *Emberiza schoeniclus*, *Carduelis carduelis*, *Passer montanus*, *P.domesticus*).

31 species of birds recorded in the domestic water basins are included in Azerbaijan's Red List, 12 of which are also included in the Red List of the International Union for Conservation of Nature. Additionally, 7 species are included only in the Red List of the International Union for Conservation of Nature.

Among water and coastal birds the *Podicipediformes*, *Pelecaniformes*, *Anseriformes* and *Charadriiformes* settle in deep and shallow water areas in winter and during migration, the *Ciconiiformes* stay in reed, sedge, reedmace and tamarisk jungles during the nesting period, while forest-bush birds stay in reed, sedge, reedmace and tamarisk jungles throughout the year.

Mammalia. The *Mammalia* class is represented with 144 species in our country. Most are spread on the land, some in the air (bats), and a small part (4-5 species) in or around the water. In this article, we will focus on some species, whose life style is related to water reservoirs (I.K.Rakhmatulina, 2005).

Family: Soricidae

Genus: Sorex L, 1758

Species: Neomys teres Satunin, 1913

It is considered as one of the least studied species in Azerbaijan.

Systematics: has changed twice. The length of the body is usually 80-100 mm, the length of the tail is 53-75 mm, the body mass is 13-25 grams. Its natural habitat covers a very large area. In Azerbaijan it can be found on the territories of Nakhchivan AR, Garabagh, Lankaran, as well as Guba district. Its population is low. *Neomys teres* is spread across different landscapes. Their lives are associated with lakes, rivers, coastlines of small rivers, swamps and the edges of ditches with permanent water supply. It swims well.

Its food consists mainly of animals that live in water. *Neomys teres* eats the food it obtained from the water on the land. It usually eats invertebrates living in water, fish caviar and younglings, and frogs. It has an active lifestyle throughout the day getting more active at night. The representatives of the species dig their setts themselves or settle in already dug ones. They give birth to 4-14 young

ones during spring-summer months. The species damages fish farms (<http://journals.tubitak.gov.tr/zoology/issues/zoo-16-40-6/zoo-40-6-1-1507-46.pdf-su>).

Family: Myocastoridae

Genus: Myocastor Kerr, 1792

Species: Myocastor coypus Molina, 1782

The *Myocastoridae* biology and ecology have been studied poorly in the natural areas of Azerbaijan. There are no changes in their systematics. The length of their body is 60 cm, the length of the tail is 45 cm, the body mass is 5-12 kg.

The *Myocastor* live a half-water life. They settle in weak flowing and stagnant lakes, in the swampy shores of the rivers and on the shores of vegetation and water-rich marshes. The species is spread in the Andean Mountains, considered to be its main natural habitat, in an altitude of 1200 meters above sea level.

It was imported to the territory of the former USSR, including Azerbaijan, from Argentina in 1930-1932 to buy fur.

Farms have been established in the areas where the *Myocastor* is widespread (Garayazi, Aghjabadi, Mingachevir, Davachi, Shamkir, Lankaran). Standard, albino and genera of other colors have been obtained in farms. On the other hand, the species is used to eradicate nutria from the lakes in order to develop fishing. Its food is comprised of plant roots, as well as rhizogenous plants, herbs, reed plants and fruits etc. In some rare cases when there is a lack of plant nutrients, it prefers animal foods. Its mainly active at night. It can reproduce throughout the year. The species gives birth two to three times a year. The gestation period lasts up to 4 months. The species gives birth to 4 to 6 young ones each time. The reproductive ability declines at the age of 3-4. Its natural habitat is limited to the South American continent. It has been acclimatized in North America, Europe, South Caucasus, Kyrgyzstan and Tajikistan. The species has been included in the systematics since its acclimatization in Azerbaijan was completed (Azərbaycanın heyvanlar aləmi, 2004).

Family: Cricetidae

Genus: Arvicola Lacepede, 1801

Species: Arvicola terrestris L., 1758

Morphological and ecological features have not been studied sufficiently. There are no changes in their systematics.

The species is one of the big rodents. The length of their body is 24 cm, the length of the tail is 15 cm, the body mass is 70-180 g. It's spread in Europe, Asia and the Caucasus. Starting from plain regions of Azerbaijan, they can spread to regions up to 3,500 meters above sea level. Two populations that differ in terms of geography, lifestyle, zones

and altitudes were selected to study cytogenetic properties. One population of *Arvicola terrestris* is distinguished for being colonial in subnival meadows of the Greater Caucasus (Guba district, Khinalig village, 2000-2300 m above sea level) and the other one for not being colonial (Bilasuvar district, sea level). Diploid chromosome number was $2n=36$. The main number of chromosomal arms in the colonial population is $NF = 72$; since 3 pairs of chromosomes have one armed (acrocentric) structures in the ones that do not have the colonial way of life, the $NF=66$. The revealed differences confirm differences between colonial and non-colonial hydrophilic populations on the genetic level (Кулиев, Кулиев, Раджабли, 1978).

The life of the *Arvicola terrestris* is associated with the lakes, the coastline of small rivers, the edges of ditches with permanent water supply, swamps and swampy meadows. Starting from the Caspian Sea they are spread to the altitude of 3000-3500 meters above sea level in Azerbaijan.

There are different opinions about how they feed. Some researchers have found the remains of other *Arvicola terrestris* in the species' setts. In their opinion, along with plant food this rodent feeds on animal food. Such feeding causes change in its economic significance. However, in the former Ryazan province only plant food was identified in the stomach's of 30 individuals. Bekstrem believes that the rodent is fed only by plant food. It only uses animal food when hungry (Azərbaycanın heyvanlar aləmi, 2004). The location of the species is easy to identify. Usually tables of food in the form of hills can be seen in the water. The species is active throughout the day.

It reproduces during the hot months of the year. The gestation period lasts for 40 days. The female individual can reproduce 3-5 times a year. The species gives birth to 6 to 8 young ones each time.

Family: Mustelidae

Genus: *Lutra* Brisson, 1762

Species: *Lutra lutra* L., 1758

***Lutra lutra meridionalis* Ognev, 1931**

The *Lutra lutra meridionalis* can be recognized for a number of signs. The length of its body reaches 60-90 cm. Its tail is 50 cm long. The mass is 6-10 kg. There is a special swimming membranes on its paws. The tail is long, muscular, and covered with short hair.

There are no changes in their systematics.

It's spread in Europe, Asia, America and North Africa. Its habitat in the Caucasus is from the Western Caucasus territories to the Lankaran Province. The northern border of the habitat is limited to Kuban and Kume. It is spread throughout the Azerbaijani part of Greater Caucasus, in different rivers of the Lankaran Natural Province. As a result of the

anthropological effects the *Lutra lutra meridionalis* are encountered in separate areas of the natural habitat, not in its entire area. *Lutra lutra meridionalis* has been recorded in 18 rivers of Azerbaijan. Density of individuals: 0.2-0.5 individuals per 10 km coastal zone. The number of *Lutra lutra meridionalis* in the country reaches 1000 individuals.

The *Lutra lutra meridionalis* usually prefers to live in mountainous and foothill rivers that have plenty of fish species (mostly salmon) and flow rapidly. It can be found in rivers located at 2000 meters above sea level. Its biology has been studied poorly. Its food consists mainly of river salmon. The species is mostly active when it's getting dark and at night.

It starts to reproduce in the beginning of winter. The gestation period lasts for 9-10 months. It gives birth to 2-4 young ones (Azərbaycanın Respublikasının Qırmızı Kitabı, 2013, Kasumova, Askerov et al., 2009, <http://www.peterlife.ru/funoffice/redbook/191963.html#.WdkSULXwHcs-çay>).

Family: *Suidae*

Genus: *Sus* L, 1758

Species: *Sus scrofa* L., 1758

***Sus scrofa atilla* Thomas, 1912**

There are no changes in their systematics.

The height of the body reaches 1 meter. The mass of male individuals varies from 200 to 340 kg. Sexual dimorphism is clearly expressed. The species differ from domestic pigs in having a more muscular body, relatively longer face, bigger canine tooth and a developed pelage. Its ears are long and wide. The eyes are small and located in the depth of the eye sockets. The tail is thin, short (14-35 cm) and has a bunch of hair at the tip. Its legs are short, the sense of smell and hearing are well developed, while the organs of vision are weak. Individuals of the South Caucasus population are relatively small compared to those in the Carpathians, Belarus and Far East. The species reaches full development at the age of 5-6.

It mainly prefers to live in parts of lakes, wetlands, forests and reed shrubberies with abundant water. It is found in all zones of the mountains up to alpine meadows. It lives in Europe, especially in oak forests, meadows and wetlands, in the Caucasus, especially in the autumn, in the fruit forests and is mostly spread in mixed and broad-leaved forests in Central Asia and Kazakhstan. It often stays in river valleys of the mountain rivers. In the Far East it lives in both cedar forests and mixed forests.

It is one of the most widespread species. It was acclimatized in North and Central America and Argentina. The species can be found in all provinces of Azerbaijan.

Sus scrofa atilla is a species with the most active feeding properties in the *Artiodactyla* fauna

settled in Azerbaijan. It feeds on everything. When the species experience food shortages in places where they live, they make long-distance migrations (60-70 and sometimes 80-100 km). The most abundant food is in the summer (34 species), and the poorest diet is in the winter season (16 species). It should be noted that the food ration of the *Sus scrofa atilla* in Azerbaijan include 68 species of plants belonging to 26 families, as well as 10 species of animal food.

Females usually start to reproduce in the second year of their development, while males do it at the age of 4-5. The gestation period lasts for 124-140 days, 130 days on average. The gestation period in females that reproduce for the first time is shorter. The weight of newborn young ones is 600-1650 grams, 850 grams on average.

Wild boars have been completely eradicated in the Lesser Caucasus natural province by the 1930s (Safarov, 1959, Sokorov, 1959). At present, no individuals are left in the occupied Nagorno-Karabakh region and its surrounding districts (excluding Goygol MP). Formerly the areas where the *Sus scrofa atilla* were most spread were regions in the Kura-Araz natural province. Although there were plenty of boars in the stagnant water areas around Kura, most of these biotopes are now farmland and animals are very rarely found there. There used to be numerous boars in the territory of Lankaran lowland-geographical district of Lankaran natural province. At present, the biotopes, which have a certain number of this species (Hirkan National Park and Hirkan Reserve, the Gizilagaj Park and the Gizilagaj Reserve), are protected (3,6).

To sum up, in more than 80 years since the establishment of the Institute of Zoology our zoologists have recorded more than 1000 species of unicellular organisms, over 1350 species of invertebrates, 190 species of vertebrates and 332 species of fish parasites (more than 100 of which are unicellular) in the flowing and stagnant water reservoirs of our country. However, the registration of new hydrobionts in future is not excluded. Because there are still many species among the aquatic animals that are not fully studied or are very poorly studied. Along with Protozoa this includes worms, mollusca, arthropods, *Plecoptera*, *Trichoptera*, *Hydrachnidia*, *Trypanosomatidae*, larvae of dipterous insects and representatives of other phyla.

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Azərbaycanın Daxili Su Ekosistemlərinin Bioloji Müxtəlifliyinə Dair

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Məqalədə ölkəmizin daxili su tutarlarından (bulaqlar, çaylar, su anbarları, göllər və s.) ayrı-ayrı tədqiqatçılar tərəfindən indiyə qədər qeydə alınan qruplar üzrə növlərin sayı təqdim olunub. Su tutarlarımızda ən geniş yayılmış və dominant növlər, onların ekosistemdə rolu və s. haqqında məlumat verilir. Ölkəmizdə elmi-zooloji tədqiqatların aparıldığı ilk vaxtlardan bu günə qədər su tutarlarımızda 1000 növdən çox ibtidai bir-hüceyrəli heyvan (əsas yerdə kirpikli infuzorlardır), 1350 növdən çox onurğasız heyvan [əsas yerdə rotatorilər (300 növdən çox) və həşərat sürfələri (586 növdən çox)], 190 növ onurğalı heyvan (əsas yerdə quşlar (97 növ) və balıqlardır (67+1 növ) və 332 növ balıq parazitləri [(əsas yerdə miksozoqlar (59 növ), yastı qurdlar (69 növ) və trematodlardır (81 növ)] qeydə alındığı vurğulanır. Hidrobiontların növ sayını bildirən bu rəqəmlərin çox güman ki, gələcək tədqiqatlarda dəyişilməsi də istisna deyil, çünki hidrobiontlar arasında elə qruplar vardır ki, onların növ tərkibi ya heç öyrənilməmiş, ya da tədqiqatlarda cəmi 1-2 növ təsadüfən qeydə alınmışdır. Buraya birhüceyrəli heyvanlarla yanaşı kirpikli qurdlar, nematodlar, xərçəng-kimilər, astagəzənlər, bulaqçı, baharçı və gündəca sürfələri və digər həşərat sürfələri daxildir.

Açar sözlər: Azərbaycanın su tutarları, biomüxtəliflik, Protozoa, onurğasız heyvanlar, onurğalı heyvanlar

К Биологическому Разнообразию Внутренних Водных Экосистем Азербайджана

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В статье представлено, зарегистрированное на сегодняшний день отдельными исследователями количество видов по группам, обитающим во внутренних водоемах страны (родники, реки, водохранилища, озера и т.д.). Предоставляется подробная информация о наиболее распространенных и доминирующих видах в наших водных ресурсах, их роль в экосистеме и т.д. С начала научных и зоологических исследований и по сей день в нашей стране отмечено более 1000 видов простейших животных (в основном, ресничные инфузории), более 1350 видов беспозвоночных животных (в основном ротатории, которые представлены более, чем 300 видами, и личинки насекомых - более 586 видов), 190 видов позвоночных (в основном, птицы - 97 видов) и рыбы (67 + 1 вид) и 332 вида рыбных паразитов (в основном, миксоzoи - 59 видов, плоские черви - 69 видов, и трематоды - 81 вид). Цифры, показывающие видовое количество гидробионов, возможно будут изменены в результате будущих исследований, так как некоторые группы гидробионтов или все еще не изучены, или в исследовании случайно зарегистрированы только 1-2 вида. Кроме одноклеточных животных сюда можно отнести ресничных червей, нематод, ракообразных, тихоходок, поденок и прочих личинок насекомых.

Ключевые слова: Водоемы Азербайджана, биоразнообразие, Protozoa, беспозвоночные животные, позвоночные животные

Spreading of Scarab Beetles (*Scarabaeidae*) Distributed in Azerbaijan Territories of the Greater Caucasus in Ecological and Zoogeographical Groups

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The material was collected during 2013-2015 years in the agrocenoses and biocenoses of the Guba-Khachmaz, Sheki-Zagatala and Absheron physical-geographical regions of the Greater Caucasus Natural Area. Analysis of the materials showed that 57.4% of the species common for studied territories are arexerophilous, 40.74% are mezophilous and 1.85% are eurybiont. According to zoogeographical analysis 3 species (5.55%) belong to Transpalearctic group, 1 species (1.85%) to Eurosiberian group, 9 species (16.67%) to European group, 5 species (9.26%) to Steppe-lowland group, 34 species (62.98%) to Mediterranean group and 2 species (3.7%) to Middle Asian group.

Keywords: *Scarabaeidae*, ecology, zoogeographic groups

INTRODUCTION

Every year pests cause great damage to agricultural plants decreasing the level of their productivity and the quality of products. That is why the detail studying of pests and effectively controlling of their number is very urgent.

Coleoptera is the most numerous and rich in species composition order of *Insecta*. From 83 species of *Melolonthinae* (*Scarabaeidae*) distributed in Azerbaijan about 44 species are the serious pests of agricultural plants. During last 40-50 years some authors (A. Atakishiyeva, T. Mamedova, N. Samadov, A. Salmanov, etc.) carried out researches on spreading and damage of some species in the different regions of the republic (Mamedov and Atakishiyeva, 2010). However, there is not enough information about biology of *Melolonthinae*, their trophic relations, seasonal activity and ecological factors affecting them. The *Scarabaeidae* family have not been studied ecologically and zoogeographically.

MATERIALS AND METHODS

The material was collected in different agro- and biocenoses of the Guba-Khachmaz, Sheki-Zagatala regions and Absheron peninsula in 2013-2015. Researches were carried out according to standard entomological methods (Narimanova and Ahmadov, 2016). Zoogeographical characteristic was given according to G.M. Abdurahmanov's method (Abdurahmanov, 1983).

RESULTS AND DISCUSSION

Most of scarab beetles are related to plant associations. Some species inhabit humid forests, other species prefer deserts and hemi-deserts.

Species collected by us in the Azerbaijan territories of the Greater Caucasus belong to the following ecological groups.

Xerophile species- include species living in steppes, semideserts, dry foothills, mountains, sunny mountain slopes, etc. These species usually occur at the height of 400-1800 m above sea level. The xerophile species make 57.4% (31 species) of all species (54 species).

Mesophile species populate steppes, foothills and forests. They can occur at the height of 1500 a.s.l. This group includes 22 species making 40.74% of total species (Fig. 1).

Eurybionts include only one species. It is *Amphimallon solstitialis* Linnaeus, 1758 the representative of subfamily *Rhizotroginae*. It lives under different ecological conditions in steppes, mountains, foothills, natural and agrocenoses. It is found at the height of up to 1800 m a.s.l. and gives 1 generation in a year.

The Greater Caucasus Natural Area of Azerbaijan has a favorable condition for development of scarab beetles. Therefore, *Scarabidae* in Azerbaijan is rich in species composition.

Azerbaijan is a part of South Caucasus and included in the Western Mediterranean Province of the Mediterranean subregion of Palearctic ecoregion.

Species collected in the studied area can be distributed between zoogeographical groups below (Figure 2):

1. Transpalearctic species; spread widely, up to North Taiga boundary. Boreal Transpalearctic subregion of this ecoregion is represented by 2 species (*Trichius fasciatus* Linnaeus, 1758 and *Potosia metallica* Herbst., 1782).

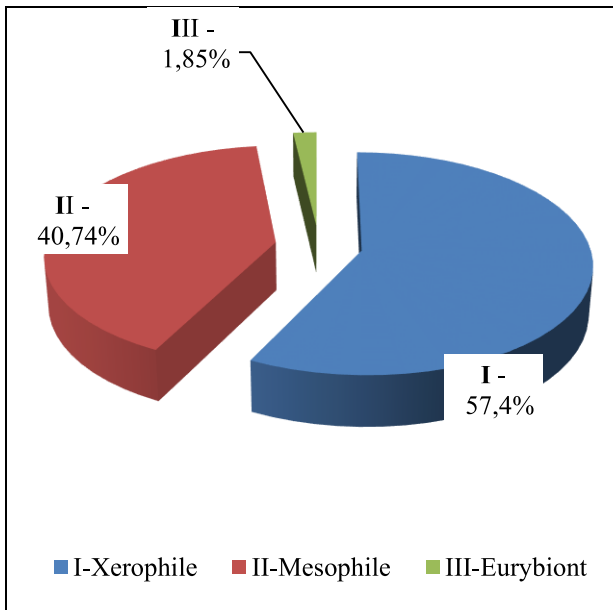


Figure 1. Distribution of scarab beetles by ecological groups.

South Transpalearctic subregion includes species distributed in the Central Asia, Pribaykalye, Mongolia, South Primorye and some areas of Siberia. The subregion is represented in Azerbaijan by *Valgus hemipterus* L., 1758.

2. Eurosiberian species: The species are widely distributed mainly in the territory of Western Europe till Eastern Siberia and Mongolia. From this region one species only (*Cetonia aurata* Linnaeus, 1758) is founded in the Greater Caucasian Natural Area of Azerbaijan.

3. Euromediterranean species of the European zoogeographical region are distributed widely in North Africa included Kazakhstan and Central Asia. Studied area represents *Oxythyrea funesta* Poda, 1761 only.

The representatives of the South-European subregion of the European zoogeographical region are mainly distributed in Ukraine, Crimea and Caucasus.

Epicometis hirta Poda, 1761 and *Potosia affinis* Ander., 1791 from this subregion regorded in the studied area.

Melolontha pectoralis Germ., 1854, the species of the Central Eurocaucasian subregion of the European zoogeographical region is recorded in the Sheki – Zagatala and Absheron regions.

4. Species from Steppe (lowland) zoogeographical region are mainly distributed in the European and Asian steppes, sometimes they occur in the Mediterranean territory. *Anisoplia segetum* Herbst., 1873 and *Pentodon idiota* Herbst., 1789 the species distributed in the Western Steppe subregion (the spread of this species in the territory from

Middle Europe to Caucasus and West Kazakhstan) were recorded in the studied area. The second subregion of the Steppe region is characterized by widespread species. Their area covers the territory from Central Europe to Eastern Kazakhstan steppes and Central Siberia.

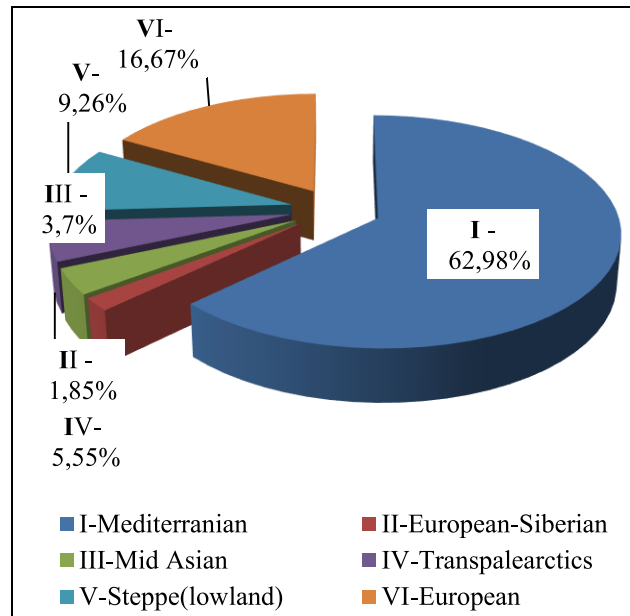


Figure 2. Distribution of scarab beetles by zoogeographical groups

5. Species from Mediterranean-Central Asian-Indian subregion of the Mediterranean zoogeographical region are widely distributed in the territory of Mediterranean sea, Central Asia, Iran, Pakistan and India. *Oxythyrea cinctella* Schaum., 1841 from this subregion is recorded in the studied area.

From Mediterranean-Caspian-Iranian subregion the *Rhizotrogus aestivus* Ol., 1789 and *Sericina caspia* Fald., 1837 are distributed in the Greater Caucasus.

In the studied area the Eastern Mediterranean species *Polyphylla olivieri* Cast., 1840 is recorded in the Guba-Khachmaz, Sheki-Zagatala and Absheron regions, *Amphimallon caucasius* Gyll., 1817 and *Potosia cuprina* Motsch., 1849 in Sheki-Zagatala region only. *Amphicoma arctos* Faldermann, 1835, *A. psillotrichia*, *Anisoplia leucaspis* Gast., 1840, *Amphicoma bombylifformis* Pallas., 1781, *Anoxia pilosa* Fabricius, 1792, *Miltotrogus aequinoctialis* Herbst, 1790 and *Maladera punctatissima* Fald., 1834 are recorded in the different regions.

Species from this subregion are widely distributed in Balkans (sometimes in Italy), the European part of the former USSR up to Caucasus, Iran, Western Kazakhstan and Central Asia.

The following species belong to Southwest Asiansubregion of the Mediterranean geographical region: *Anisoplia signata* Fald., 1835, *A.faldermanni* Reit., 1883, *Amphimallon solstitialis setosus* L., 1758, *Epicometis suturalis* Reitter., 1913, *Potosia speciosa* Adams, 1817, *P.hungarica* Herbst., 1832. These species are distributed from Southwest Asia to Caucasus, North Iran, sometimes Turkmenistan.

Oryctes nasicornis L., 1845, *Blitopertha lineata* Fabr., 1798, *Potosia hieroglyphica* Men., 1832 belong to Caucasian-Iranian subregion. These species are frequently found in South Turkmenistan.

Oxythyrea albopicta Motsch., 1845 belongs to Caucasian- Central Asian subregion, however, *Rhizotrogus serrifunus* Mars., 1879, *Anisoplia austriaca* Hrbst., 1783, *Epicometis senicula* Men., 1832, *Anomala abchasica* Motsch., 1854, *Anisoplia signata*, *A.farraria* Er., 1847, *Adoretis discolor* Fald., 1835, *Melolontha aceris* Fald., 1835, *Hoplia caucasica* Medvedyev, 1952, *Potosia asiatica* Fald., 1835, *Homaloplia adulta* Reitt., 1887 to Caucasian subregion.

6. Two species *Adoretus nigrifrons* Stev., 1809 and *Polypylla adspersa* Motsch., 1853 represent the Central Asian zoogeographical region.

Analyses of the materials show that 3 species (5.55%) belong to Transpalearctic, 1 species to Eurosiberian (1.85%), 9 species to (16.67%) European, 5 species (9.26%) to Steppe (lowland), 34 species (62.98%) to Mediterranean and 2 species (3.7%) to Central Asian group.

Collected material was analyzed according to distribution among regions of the Greater Caucasus and the following results have been obtained:

Anisoplia farraria, *A.faldermanni*, *A.signata*, *Hoplia caucasica*, *Homaloplia adulta*, *Gnorimus subcostatus*, *Oxythyrea albopicta*, *Potosia cuprina*, *P.speciosa*, *P.affinis* were recorded only in the Sheki-Zagatala region.

Epicometis suturalis was recorded in the Guba-Khachmaz region.

Pentodon bidens Pall, 1771, *Polyphylla alba* Pall 1773, *Anomala proticola*, *Sericina caspia*, *Chioneosoma pulverum* Knoch., 1801, *Potosia asiatica* Fald., 1835, *Anisoplia segetum* were recorded only in Absheron.

Oryctes nasicornis, *Pentodon idiota*, *Polyphylla olivieri*, *P.adspersa*, *Anoxia pilosa*, *Adoretus discolor* Fald., 1835, *A.nigrifrons*, *Anomala errans*, *A.abchasica*, *Blitopertha majuscula* Medvedyev, 1949, *B.lineata*, *Ansioplia leucaspis*, *A.austriaca*, *Amphicoma vulpes* Fabricius, 1781, *Valgus hemipterus*, *Amphimallon solstitialis*, *Miltotrogus aequinoctialis*, *Epicometis hirta*, *E.senicula*, *Oxythyrea funesta*, *O.cinctella*, *Cetonia aurata*, *Potosia hieroglyphica* and *P.hungarica* were recorded in all three regions of the Azerbaijan territory of the Greater Caucasus.

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**Azərbaycanın Böyük Qafqaz Təbii Vilayətində Yayılmış Lövəbəbiğ Böcəklərin
(Scarabaeidae) Ekoloji və Zoocoğrafi Qruplar Üzrə Paylanması**

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Məqalədə Azərbaycanın Böyük Qafqaz təbii vilayətindən toplanmış lövhəbiğlər fəsiləsinə məxsus 54 növ xırıladağ böcəyin ekoloji və zoocoğrafi qruplar üzrə paylanması və fiziki-coğrafi rayonlar üzrə yayılması haqqında məlumatlar verilir. Materialların təhlili göstərirki, Böyük Qafqazın Azərbaycanın ərazisində yayılmış 54 növ lövhəbiğ böcəklərin 57,4%-ni kresofil, 40,74%-nimezofil növlər təşkil edir. Evribiont növlərdən isə yalnız 1 növə rast gəlinir. Zoocoğrafi vilayətlər üzrə Transpaleartikaya 3 növ (5,55%), Avropa-Sibir vilayətinə 1 növ (1,85%), Avropa qrupuna 9 növ (16,67%), Çöl (düzənlik) vilayətinə 5 növ (9,26%), Aralıq dənizinə 34 növ (62,98%), Orta Asiya qrupuna isə 2 növ (3,7%) daxildir.

Açar sözlər: Scarabaeidae, ekologiya, zoocoğrafi qruplar

**Распределение по Экологическим и Зоогеографическим Группам
Пластинчатоусых Жуков (Scarabaeidae), Распространенных
на Азербайджанской Территории Природной Области Большого Кавказа**

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В статье приводятся данные о распределении по экологическим и зоогеографическим группам 54 видов пластинчатоусых жуков, собранных из районов Азербайджанской территории Большого Кавказа. Анализ материалов, показывает, что из 54 видов пластинчатоусых жуков, распространенных на территории Большого Кавказа Азербайджана, 57,4% составляют ксерофилы, а 40,74% - мезофиллы. Из эврибионтов можно встретить лишь 1 вид. По зоогеографическим областям 3 вида относятся к Транспалеарктике (5,55 %), 1 вид - к Европейско-Сибирской области (1,85%), 9 видов - к Европейской группе (16,67%), 5 видов - к степной (равнинной) области (9,26%), 34 вида - к Средиземному морю (62,98%), 2 вида - к Средне-Азиатской группе (3,7%).

Ключевые слова: Scarabaeidae, экология, зоогеографические группы

The Adaptive Abilities of the Natural and the Hatchery Juvenile of Kura Sturgeon (*Acipenser Güldenstadti Persicus Natio Kurensis Borodin*) to Various Environmental Factors in Early Ontogeny

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The present study explores the effects of the growth conditions on food-getting and protective behavioral skills of the juvenile fish of Kura sturgeon (*Acipenser guldenstadti persicus natio Kurensis Belyaev*) grown in hatchery for 70 days and the wild juvenile fishes of the same age caught in the Kura river. Especial stress is placed on identification of the most sensitive periods of early ontogenesis in development of those vital behavioral responses. It is recommended to release juvenile fish from hatcheries in earlier periods of ontogenesis.

Keywords: Living conditions, critical periods of development, keeping of the juvenile fish in ponds

INTRODUCTION

Biodiversity conservation is among the top priorities of the modern science. In recent years there has been a dramatic decrease in abundance of the most ancient fish species – sturgeons in the Caspian Sea.

The dramatic decline in the stocks of sturgeon in natural water bodies is caused by a number of factors including the uncontrolled poaching of the fish irrespective of the age and size, contamination of the fish natural habitats, reduction in effectiveness of natural spawning, decrease in numbers of juvenile fish released from hatcheries etc. (Baranikova et al., 2000; Gerbilsky, 1967; Gorbunov et al., 2002; Hadjiyev, Kasimov, 2005; Ivanov, 2000; Kasimov, 1980; Vlasenko et al., 2002).

At present time, the main area of accumulation of sturgeons in the wild is the Caspian Sea. The majority species of this family are the anadromous fish, that spend most of their life in sea waters before travelling to breed in the upper reaches of rivers, while the offspring born in river waters slip back into sea waters and live there up to the age of sexual maturity.

Since the 50s of the last century, the natural reproduction rate of sturgeon has remarkably reduced as a result of construction of hydraulic facilities on the fish spawning routes in the rivers. At the present time, the natural reproduction of sturgeons occurs only in small areas downstream of those structures.

In order to compensate for the loss of natural spawning grounds in the lower reaches of rivers,

sturgeon hatcheries have been built in which eggs gotten from mature sturgeon species are cultivated in hatcheries up to the so-called viable age (2-3 months), after which they are released into natural reservoirs to maintain the natural stocks of the fish. The main idea of fish reproduction in hatchery conditions is to get more offspring and to release larger number of juvenile fish into natural water bodies. By now a great number of dedicated research has been conducted to study the morpho-physiological and biochemical indices of hatchery-grown juvenile fish (Kokoza, 1971; Kokoza, Lukyanenko, 1970; Korzhuev, 1967; Krayushkina, 1968; Lagunova, 1981). However, the survival rate and the level of adaptation of hatchery-grown juvenile fish in natural conditions are poorly understood (Makhmudbekov et al., 1966; Soldatova, 1968; Vodovozova, Kasimov, Soldatova, 1974). Furthermore, the degree of adaptation to particular conditions of rivers of juvenile fish has practically not been taken into account. Probably for this reason, the supposed commercial profit of sturgeons to the released juvenile fish appeared to be by 100 and 1000 times lower than expected.

The point is that, while identifying the period of time required for cultivation of juvenile fish in hatcheries, some important biological characteristics of the fish and the specific environmental conditions of particular region were not fully taken into account including the development of vital behavioral responses ensuring survival in the wild. According to literature data (A.N. Derzhavin, 1956; Ginzburg, 1957; R.V. Hajiyeve, R.Y. Kasimov, 2005), in the wild, the juvenile sturgeon slip into the Kura river

from the natural spawning areas at age of larvae or when the body weight is between 50 to 800 mg. The bigger individuals rarely occur in catches. Therefore, it was important to carry out the comparative study of the adaptation potential of wild and hatchery-grown juvenile sturgeon of the same age.

It is well known that the protective and food-getting responses play an important role in adaptive reactions of organism, which largely depend on the environmental conditions. In this regard, it was necessary to study the degree of manifestation of these responses in hatchery and wild juvenile fish of different ages comparatively, which would allow to reveal the influence of environmental conditions on development of those responses in early periods of ontogenesis. For this reason, we have studied the degree of formation of protective and food-getting behavioral responses and the effect of environmental conditions on these responses in juvenile sturgeons of different age grown in the hatchery and in the wild. In addition, the resistance to starvation of juvenile sturgeons of natural and artificial generation was studied in order to identify the adaptive potential of juvenile fish grown in the hatchery as compared to those grown in the natural conditions.

MATERIALS AND METHODS

The present study was carried on larvae and juvenile specimen of sturgeon (*Acipenser güldenstadti persicus natio Kurensis Belyaev*) of different age and size, grown in the hatchery conditions and caught from the Kura river.

The hatchery-grown specimen were taken from the pools of the Kura Experimental Sturgeon Fish Hatchery. The wild juvenile fish was caught in late May - early June with the trawl net employed from motor boat. The caught specimen was examined to identify the species identity, and to measure the body length and weight.

Trawl caught was conducted at 40-50 km upstream the mouth of Kura river, near Uzunbabaly and Abbasaly villages of Neftchala region of Azerbaijan, in distance of 10-15 km away from the Kura Experimental Sturgeon Fish Hatchery. The areas were selected because normally no hatchery juvenile fish occur in those areas since the juvenile fish released from hatcheries cannot reach those sites. In that particular stage of the development the juvenile sturgeons manifest the instinct to slip downstream in the estuary. Therefore, all the sturgeon larvae and juvenile fish which were caught in that part of the river, were the offspring of natural spawning. In total, trawling caught was conducted 10 times during 3 days. The duration of trawling was 20-35 minutes. In total, 44 specimen of juvenile fish of wild generation were caught.

The caught larvae and juvenile fish were placed into special pans and were transported to the experimental plant where they were replaced into pools with the running water and were grouped into three age groups in accordance with their body size and weight (Table 1).

The wild juvenile sturgeon species were placed into the pools for adaptation for 4-5 days. They were fed daphnia (*D. magna*) and white flour worms - oligohetams. The hatchery-grown fish was given the same food. The food-getting behavioral reactions of juvenile sturgeons were studied under conditions of free movement in circular pans. The diameter of each pan was 80 cm (Fig. 1).

Two pans (**A** and **B**, see Fig. 1) connected by a passage were used in experiments. The left pan (**A**) was divided into two parts by a partition (**a** and **б**). The partition has a door so that the fish can move from one part (**a**) to another one (**б**). In non-experimental days the fish were fed in the pan **B**.

In that pans, were placed the fish which were out of the experiment, they did not see experimental fishes as the partition was non-transparent.

Experiments were carried out according to the following schemes: every specimen was taken through the door (**3**) from the part "**a**" into the part "**б**". The fish were not fed in the day of experiment. In order to ensure the development of conditional feeding reflexes it was necessary to ensure that the juvenile fish swim to another pan (**B**) and approach the point of conditional feeding (**Г**). When the juvenile fish were approaching the point they were given 2-3 worms and simultaneously a conditional stimulus was presented (light illumination of 40 lux) (Fig. 1).

During the experiment, the conditioned illumination stimulus was followed by the food supply. Eventually the conditioned stimulus was presented at the time when the appeared in the part "**б**" (pan **A**).

The manifestation of conditioned food-getting reflex was recorded when the fish in the part "**б**" (pan **A**) responded to conditioned stimulus and moved towards the point of food supply (pan **B**). A stable conditioned food reflex was recorded if the juvenile fish repeatedly (by 5-6 times) responded to the conditioned stimulus and swam to the feeding point.

During the experiment the conditioned stimulus was presented 7-8 times with interval of 2 minutes. The duration of provocation was 15-30 seconds.

The manifestation of defensive response to predator was studied by placing a few fish specimen to area with predator (river perch). The fish behavior was monitored during 30 minutes (Fig. 2).

Table 1. Body weight and length of the juvenile fish of different generations.

Growth conditions	Age (days)	Fluctuation range	
Hatchery	18-20	140-150	2.8-3.0
	28-35	350-360	4.5-6.1
	50-55	720-1.100	6.2-7.0
	70-80	3.900- 4.500	13.9-14.0
Wild	18-20	90-130	2.9-3.0
	28-35	280-310	4.3-6.2
	50-55	610-900	6.2-6.9
	70-80	—	—

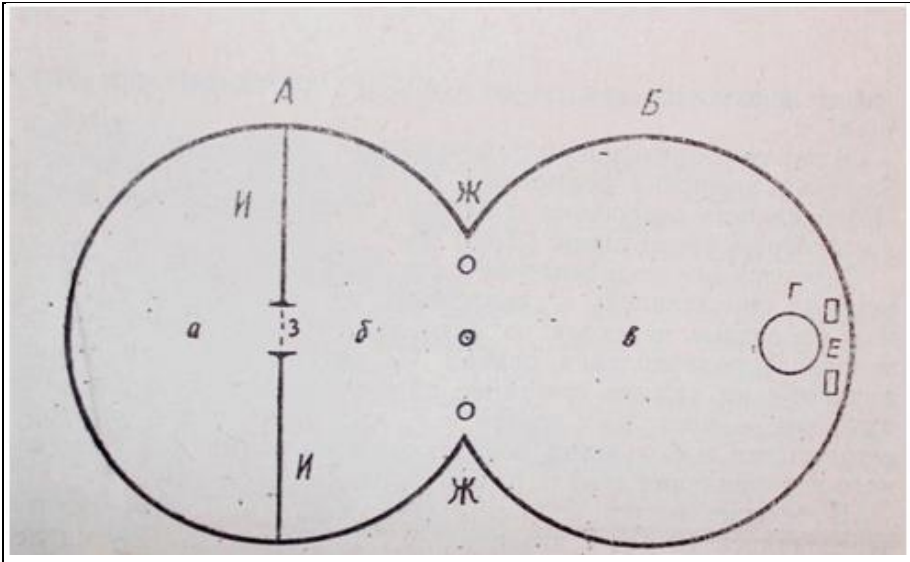


Fig. 1. Scheme of experemental piscicultural pans for development of food conditional responses in the sturgeon larvae and fry

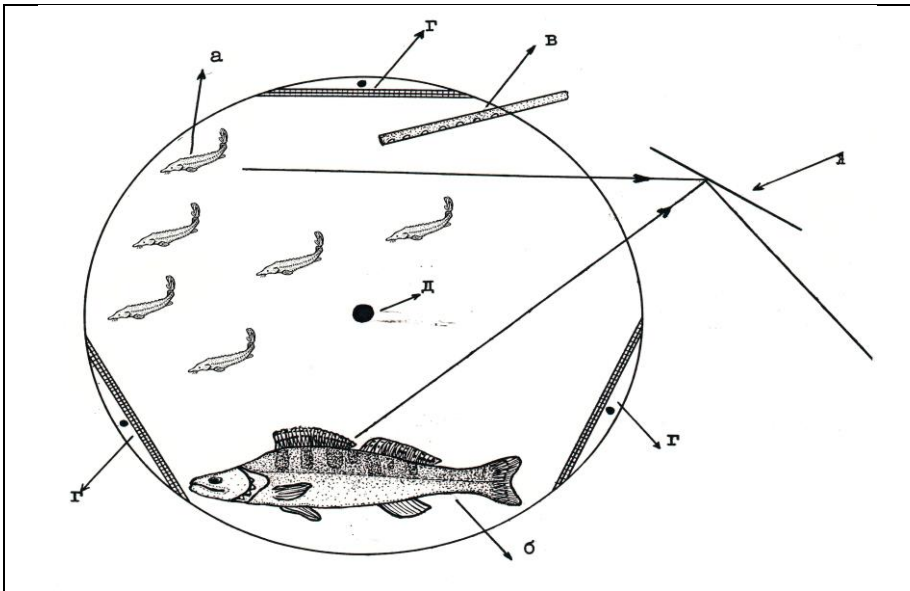


Fig. 2. The fish behavior monitoring during 30 minutes.

Also, was recorded the time of avoidance and hiding of juvenile fish in the presence of predator in their surroundings. To one pan were placed 2 specimen of perch (of sizes of 22 and 36 cm) and 10 specimen of wild or hatchery juvenile fish of the

same age. On the next day, i.e. 24 hours later the number of survived juvenile fish was recorded. In total, 4-5 series of experiments were carried out with each age group.

RESULTS AND DISCUSSION

The findings of the present study show that, at the age of 18-25 days, both the hatchery and wild juvenile sturgeons are able to develop the conditioned responses to combination of a neutral stimulus (e.g. light illumination) and biologically important stimulus (food) (Fig. 3). At this age, the hatchery-grown juvenile fish develop the food-getting skills even faster than the wild ones. One possible explanation is the high level of development of orientation responses in the hatchery-grown fish. As to wild juveniles, our point is that the new surroundings causes agitation and anxiety in the wild juvenile fish placed in artificial conditions of pans and it takes some time for the wild fish to get adapted to new conditions.

At the age of 30-35 days, the conditioned food reflex in juvenile fish of both the wild and the hatchery generations is developed faster as compared to the age group of 18-25 days. In the age group of 30-35 days, no differences were recorded between the wild and the hatchery juvenile fish with regard to the rate of formation of the conditioned food responses.

At the age of 50-55 days, the significant differences in the rate of formation of food conditioned responses were recorded between the wild and the hatchery juvenile fish. In particular, the wild juvenile fish develop the responses by 2 times faster as compared to the hatchery species. Also, in hatchery juvenile fish of the age of 50-55 days, the formation of the conditioned food responses required more time as compared to the hatchery juvenile fish of earlier ages (Fig. 3).

We suppose that the abovementioned differences are associated with the habitat conditions in the natural and artificial environments, which are reflected in development of the fish nervous cells.

It should be noted that there were differences in manifestation of the protective responses between the wild juvenile sturgeon placed into the hatchery pools, and the hatchery juvenile fish of the same age. To identify the nature of these differences we conducted the comparative study of the behavior patterns of the wild and hatchery juvenile fish in presence of predators. As predator we used the river perch (*Stizostedion lucioperca*) of 22-36 cm. The findings revealed that, at the age of 18-25 days, there were no difference in the survival rate between the hatchery and the wild juvenile sturgeon. Piscicultural pans were used to study the behavior of juvenile fish of different generations in the presence of predator: а - young sturgeons; б - predator; в - tube for water supply; г - source of the conditioned stimulus -light illumination; д - tube for outflow of water; and ж - mirror for observation (see Fig 2).

As to the next age group of 40-35 days, during 24 hours the predator managed to catch only 1-3 specimen of the wild fish (50% in average), while this figure was about 20% with regard to the hatchery fish.

Important to note that at the age group of 50-55 days, no case of catch by predator of the wild specimen was recorded while in average 30% of the hatchery juvenile fish got eaten by predator.

At the age of 70-80 days, 25% of the hatchery sturgeon got eaten by predator. The juvenile fish of natural generation of this age were not recorded in the river catches, because normally at this age the juvenile sturgeon move back to the sea waters.

The findings of these experiments allow to conclude that starting from the age of 50-55 days, there is a remarkable difference between the wild and hatchery juvenile sturgeon in manifestation of defensive responses to presence of predator. Specifically, while the wild fish respond to presence of predator by hiding or by rapid swimming away, the hatchery juvenile sturgeon remain neutral to the predator's presence, sometimes even swim towards the predator eventually getting into the jaws (see Fig. 2).

The findings of observations of the food-getting activity of the hatchery and the wild sturgeon in the studied age groups reveal no difference for the age groups of 18-20 days and 28-35 days, while at the age of 50-55 days the wild fish find the food by 2 times faster as compared to the hatchery fish (see Table 2).

Analysis of the data presented in Table 2 shows that at the age of 18-20 days the fish of both generations need more time to find the food as compared to those at the age of 28-35 days.

One of the distinguishing indicators of habitat of the hatchery and the wild juvenile sturgeon in the Kura river is turbidity of water. Waters of the Kura river becomes muddy after getting the flow from the Araz river. It should be noted that the hatchery-grown juvenile sturgeon that live in clean water up to release from hatcheries, after the release find themselves in the river waters where the visibility is practically equal to zero. The wild juvenile sturgeon which get out from eggs under the Mingec-haur reservoir live in clean waters for a few days and then slip downstream into the muddy waters. In areas, where hatcheries release the juvenile fish, the water is muddy. Therefore, the comparison of the behavior of hatchery fish with the wild ones in muddy water was important in order to find out the influence of water conditions upon the development of vital functions of the fish.

The findings of our laboratory research dedicated to the comparative study of behavioral patterns of the wild and the hatchery juvenile sturgeon at different ages, show that, irrespective of the age, the juve-

nile fish grown in the hatchery, after being transplanted from clean water to muddy water, remain motionless for 60-100 seconds. They do not respond to tactile stimulation, switching of light and other provocations. Only after 2-3 minutes the fish start taking slow swimming movements in different directions. On the 8-10th minute the movement and orientation in the space get normalized, but juvenile fish do not always respond to knocking on the aquarium and to change of light conditions. At the same time, those juvenile specimens that remain in clean water, adequately respond to knocking and changing of the light conditions. In general, the behavioral responses of the hatchery juvenile fish transplanted from the clean water to the muddy water get normalized only after a day.

With consideration of the change in behavioral responses of the hatchery juvenile fish in muddy water, it was important to study the intensity of feeding of the hatchery and wild juvenile fish in muddy and clean water.

The findings of our research showed that the intensity of feeding of the hatchery and the wild

juvenile fishes significantly decreased in muddy water (see Table 3).

The findings show that the replacement of the hatchery juvenile fish from the clean to the muddy water affect to a considerable extent the intensity of feeding, resulting in its rapid decrease, as compared to the wild juvenile fish in the same conditions. One possible explanation of this difference is that the wild juvenile fish while in the river waters can encounter the turbidity conditions of water while the hatchery sturgeon live in the clean water from the very first days up to the release to the river waters. Therefore, the conditions of muddy water for the hatchery juvenile fish are more significant provocation than the clean waters for the wild juvenile sturgeon.

Our findings allow to suggest that the hatchery juvenile sturgeon, getting into the muddy river waters, may starve for some time and even die due to break-up of the food-getting activity. Therefore, it was important to explore the resistance of the hatchery juvenile sturgeon to starvation.

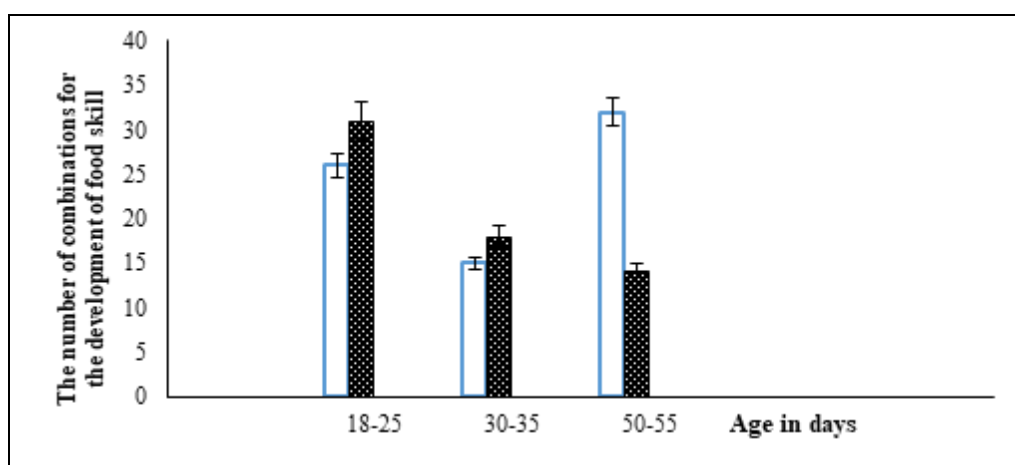


Fig. 3. Development of food skills in juvenile of sturgeon: □ – hatchery; ■ – wild.

Table 2. Food-getting activity of hatchery-grown sturgeon at different ages.

Age, days	Length, mm	Averages in seconds ($m \pm p$) of food finding by juvenile sturgeon ($p=5$)	
		Wild	Hatchery
18-20	2.8-3.0	42.40±6.18	47.90±5.95
28-35	4.4-6.0	34.20±3.59	32.50±1.98
50-55	6.2-6.8	16.20±2.04	39.60±3.11
70-80	12.0-13.5	-	35.20±4.23

Table 3. The intensity of feeding of the wild* and the hatchery juvenile sturgeon in clean and muddy water.

Age, days	Length (mm)	The average amount of food eaten (mg) in 2 hours by 5 juvenile fishes of sturgeon (experiments were carried out 3 times)			
		Clean water		Muddy water	
		Wild	Hatchery	Wild	Hatchery
18-20	2.8-3.0	14.60±0.88	25.30±0.42	34.60± 0.42	4.00±0.11
28-35	4.4-6.0	276.30±2.18	291.00±3.04	302.00± 1.06	14.30±1.33
50-55	6.2-6.8	312.60±3.16	424.30±1.43	545.00± 1.99	18.60±4.05

* After being caught from the river the wild juvenile fish were transplanted into the pools with muddy water and after 1 day were taken to the experiment.

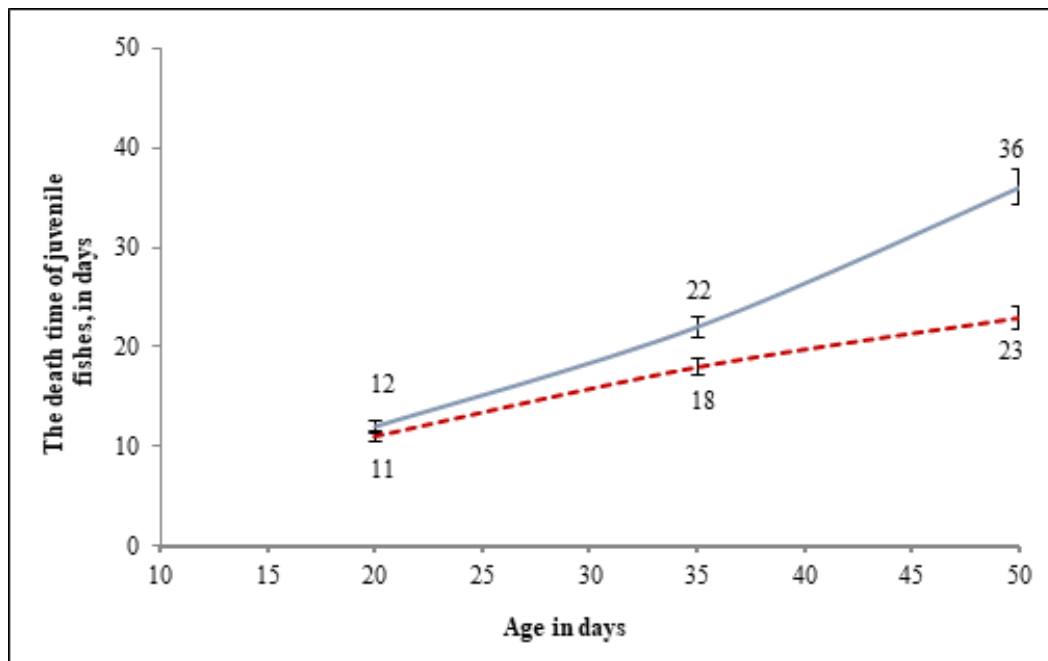


Fig. 4. The averages of death time of the wild (—) and hatchery (---) juvenile sturgeon at different ages after complete deprivation of food.

Three series of experiments have been carried out to explore the adaptive potential of the sturgeon juvenile fish of different age, both the wild and hatchery generations, to survive the full food deprivation. The findings showed that all specimen of both generations at the age of 20 days died after 11-12 days of starvation (*see* Fig. 4). The resistance of both the wild and the hatchery juvenile fish increased in the advanced age groups. In particular, the juvenile sturgeon of age of 35-50 days could survive starvation for a longer time. At the age of 35 days, the specimen of the wild sturgeon appeared to be more resistant to starvation than the hatchery ones (*see* Fig. 4). It should be noted that the same-aged wild juvenile sturgeon is less well-fed in comparison with the hatchery ones, but they are able to survive for a longer time under the conditions of the full food deprivation.

Thus, the findings of the conducted research are indicative of the differences in the level of development of the vital behavioral responses between the hatchery-grown sturgeon juvenile fish released into the natural environment of the river waters, and the wild sturgeon juvenile fish of the same age caught from the river.

We consider this as the reflection of the difference in the informational contents between the natural habitats of sturgeons and the artificial environment of the sturgeon hatcheries.

So far the biological characteristics of sturgeon species grown in hatchery and the direct influence of environmental conditions on development of the most important functions of the fish including the behavioral responses have not been fully studied.

At the same it is well known that the structural and functional development of the central nervous system in animals is largely defined by the intensity of sensory stimulation (Nikonorov, 1982; Nikonorov, Obukhov, 1983; Obukhov, Klyuyev, 1988; Vitvitskaya, 1991).

In this regard, it was important to identify the sensitive periods of early ontogenesis in the development of vital behavioral responses of juvenile sturgeon.

The findings of our earlier research reveal that the process of formation of the vital behavioral responses in juvenile sturgeons starts at the age of 18-22 days, and ends at the age of 35-45 days. By the age of 35-45 days the juvenile sturgeon manages to develop the sustainable food and protective behavioral responses (Kasimov, 1980, Kasimov, Obukhov, Rustamov, 1986; Obukhov, 1996).

The comparative study of behavioral patterns of juvenile specimen of different age of the wild and the hatchery sturgeons showed that at the age of 18-25 days there were no significant differences in the nature of manifestation of the food-getting and protective behavioral responses. Apparently, after hatching and up to the age of 18-25 days, the behavioral responses of the fish is determined mostly by the inborn reactions, while in more advanced age periods, the environmental conditions play the dominant role in the formation of both neuronal structures of the central nervous system and the most important behavioral reactions.

In more sensitive periods of ontogenesis (age of 25-55 days), the formation of the most vital functions is influenced by both the internal i.e. the

genetically inherent, and the external i.e. the environmental factors. Among the latter we should note such environment parameters as temperature, salinity, illumination, oxygen content, pH of environment, food organisms, natural enemies etc.

The findings of comparative study of food-getting and protective behavioral responses of hatchery and wild juvenile fishes of sturgeon in these sensitive stages of development show that the formation and manifestation of these responses significantly differ between the natural and artificial generation of sturgeons.

During the period of ontogenesis when the most important morpho-functional functions of the fish are developing, the juvenile sturgeon grown in the hatchery can easily find the sufficient amount of food in the surrounding habitat only in the absence of predators, while the juvenile of the same age caught from the river waters can manage finding the food in the presence of predators as well. In other words, the hatchery and natural environments differ from each other with regard to intensity of sensory stimuli. Probably, that is why the above-mentioned responses are less manifested in the hatchery juvenile fish in comparison with the wild ones.

Previously, it was shown that the cultivation of the juvenile fish in the sensory-depleted environments slows down the brain development, decreases the intensity of DNA/RNA synthesis in the neurons, affects the CNS-adaptive potential, and eventually led to change in the development rate of a number of conditioned reflexes and in ability to preserve the acquired skills (Kasimov, 1970; Kasimov, 1980; Nikonorov, etc., 1988; Vitvitskaya, 1991).

Our data suggest that, for better adaptation to the river conditions, it is necessary to release the juvenile sturgeon at the age of 28-35 days, when the morpho-functional responses of the fish are developing. This facilitates the process of formation of these functions in accordance with the conditions of the habitat, ensuring thereby the formation of behavioral responses required for better survival and adaptation to natural conditions.

More prolonged cultivation of juvenile sturgeon in the hatchery conditions (for 70 or more days) results in development of behavioral responses that ensure the survival of the fish in the hatchery conditions and those of aquaculture. In the artificial conditions of hatcheries and aquaculture, the fish are more well-fed, grow better and have high survival rates due to the better food supply and the absence of predators. However, the hatchery juvenile fish when get into the natural environment have a great loss due to difficulties in getting adapted to new conditions.

Probably, when food is enough in the surrounding environment, the metabolism processes in the hatchery juvenile fish are accelerated in comparison with the wild ones, which encounter challenges to find the food. Accordingly, the metabolism of the wild juvenile fish goes in a more efficiently way, in accordance with the conditions of their habitat.

It was shown that the biochemical indicators of caviar of the wild Black sea perches and hatchery ones differ considerably from each other (Seaborn et al., 2009). The significant differences in the level of highly unsaturated fatty acids in muscles, liver, ovaries and eggs of the wild and domesticated silver eels were also found by Japanese scientists (Ozaki et al., 2008).

The difference in various indicators between the wild and the hatchery generations of different fish species of fish are noted in other studies as well (Theriault et al., 2010; Usova, 2009; Velyansky et al., 2009; Vokota Takashi, et al., 2007).

CONCLUSION

The findings of our research allow to conclude that while cultivating sturgeon species in the hatchery conditions, it is necessary to take into consideration the biological peculiarities of development in the natural conditions, especially during the critical periods of formation of the most important physiological functions.

In this regard, especial consideration should be given to identifying the age periods, during which formation of most important morphological and functional indicators takes place that ensure the survival of the fish in a particular habitat conditions.

Our findings show, for that for the Kura sturgeon, the age of 28-45 days is a sensitive period of ontogenesis, when the most important functions of the fish organism are being formed. In this particular period of ontogenesis, the habitat conditions play especially important role. Therefore, the natural environment conditions present a number of challenges for the sturgeon juvenile cultivated in the artificial environment of hatcheries, with sufficient food and in absence of predators.

With consideration of all abovementioned, we would recommend to release the juvenile fish of the Kura sturgeon from hatcheries into the river waters at the age of 28-30 days, with the body weight of no less than 1 g., when the fish most important morphological and functional characteristics just start to get their shape, to ensure the further development of these functions in accordance with the conditions of the natural habitat. Our understanding

is that the release of juvenile fish at this age will facilitate better adaptation to natural conditions and contribute to efficiency of hatchery breeding of sturgeons in the Kura-Caspian region.

Thus, our research has shown that the natural (wild) juvenile fishes of age up to 20 days do not differ in their adaptive abilities from the hatchery ones. However, the wild fishes of older age (more than 45-50 days) differ markedly from the hatchery ones in high adaptive abilities (resistance to starvation and fluctuations in environmental factors).

We consider that in order to effectively replenish the sturgeon population in the Kura-Caspian region and to improve their gene pool, it is recommended that the natural reproduction of these fish in Kura be maintained and expanded by prohibiting fishing during their spawning in the river (May and October) to ensure the admission of producers to existing spawning grounds. During this period, it is necessary to strengthen protection measures from the mouth of the rivers to spawning grounds of sturgeon.

It is necessary to release the juvenile fishes of sturgeon, grown in the hatchery, into the river or sea at the age of 35-45 days, when food-getting and protective behavioral responses begin to form. Delay of the juvenile sturgeons in the hatchery after the age of 45 days leads to weakening of their protective and food-getting responses which are necessary for better survival in the wild (natural) environment.

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**Təbii Və Zavod Şəraitində Böyüdülmüş Kür Nərə Balığı (*Acipenser Gueldenstaedtii*
Persicus Natio Kurensis Borodin) Körpələrinin Erkən Ontogenezdə Mühitin
Müxtəlif Amillərinə Uyğunlaşma Qabiliyyəti**

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Məqalədə Kür çayından ovlanmış və zavod şəraitində nəsil alınıb böyüdülən eyni yaşlı nərə körpələrinin qida tapma və müdafiə davranış reaksiyalarını və aclığa dözümlülüyü, yaşama qabiliyyəti müqayisəli öyrənilmişdir. Məlum olmuşdur ki, 20 günlük yaşa qədər bu göstəricilərdə, təbii şəraitdən ovlanmış və süni-zavod şəraitində böyüdülmüş körpələrdə fərq müşahidə olunmur. Bu dövrdən sonra qida tapma və müdafiə davranış reaksiyaların formalaşması mühitdən asılı olaraq 30-45 günlük yaş dövründə püxtələşir və göstərdiyimiz davranış reaksiyaları zavod şəraitində böyüdülən körpələrdə, təbii şəraitdən ovlanmış eyni yaşda olan fərdlərdə daha zəif olur. Bunları nəzərə alaraq zavod şəraitində böyüdülən körpələri 30-45 günlük yaşında təbii şəraitə buraxılması tövsiyə olunur. Təbii şəraitdə böyüdülmüş körpələrin yüksək uyğunlaşma qabiliyyətini nəzərə alaraq nəəkimilərin təbii yolla nəsil verib, böyüməsini təmin etmək üçün təkliflər verilir. Bütün bunlar nərənin genofondunun qorunmasına və onların Xəzər dənizində ehtiyatlarının artırılmasına kömək edə bilər.

Açar sözlər: *Həyat şəraiti, inkişafın böhranlı periodları, körpə balıqların hovuzlarda saxlanması*

**Адаптационные Способности Естественной И Заводской Молоди Куринского Осетра
(*Acipenser gueldenstadti persicus natio kurensis borodin*) К Различным Факторам
Среды В Раннем Онтогенезе**

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В статье приведены данные о сравнительном изучении пищедобывательных и оборонительных поведенческих реакциях, а также устойчивости к голоданию и жизнеспособности у молоди осетровых, выловленных из р. Кура, и потомства рыб, которое было выращено в заводских условиях. Все изучаемые образцы относились к одной возрастной группе. Установлено, что в возрасте до 20 дней не наблюдается разницы в вышеперечисленных показателях между особями, выращенными в естественных и искусственно-заводских условиях. По истечении этого периода происходит усовершенствование пищедобывательных и оборонительных поведенческих реакций в зависимости от среды обитания, и по этой причине у осетровых в возрасте 30-45 дней, выращенных в заводских условиях, показанные поведенческие реакции бывают слабее, чем аналогичные у рыб этой возрастной группы, выловленных из естественной среды. Принимая во внимание вышесказанное, рекомендуется выпускать осетровых, выращенных в заводских условиях, в естественную среду обитания в возрасте 30-45 дней. Учитывая, что особи, выращенные в естественных условиях имеют высокую приспособляемость, в данной работе даются предложения по естественному приросту осетровых. Всё вышеизложенное может способствовать охране генофонда и воспроизводству их запасов в Каспийском море.

Ключевые слова: *Условия жизни, кризисные периоды развития, содержание молоди в водных бассейнах*

The Study of the Correlation Between Doppler Indices of Intratumoral Blood Flow and CD-31 in Malignant Ovarian Tumors of Epithelial Origin

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The correlation among quantitative indices (VI-vascular index, PI-pulsatility index, RI- resistive index, VFI-vascular flow indices) of dopplerometry with CD-31 in malignant ovarian tumors was studied. ROC examination of quantitative indices of dopplerometry was also performed in malignant ovarian tumors. As a result of the research a positive correlation relationship between dopplerometric indices of blood flow and CD-31 was established. It was found that increases in dopplerometric indices occur in accordance with CD-31 depending on the degree of malignancy.

Keywords: *Ovarian cancer, CD-31, dopplerography, neangiogenesis*

INTRODUCTION

The growth and metastasis of cancer cells is based on angiogenesis - forming and growth of new blood vessels. The formation of microvascular net is important for providing tumor metabolism (Morgan et al., 2007). Creation of new microveins occurs through vasculogenesis, intussusceptive angiogenesis and neangiogenesis. The last mechanism plays a crucial role in this process.

Migration of perisits from basal membranes of capillaries, degradation of extracellular matrix around capillaries, migration of endothelial cells, formation of tube-like structures and at last formation of anastomoses with neighboring veins occur during neangiogenesis (Carmeliet, 2005; Emoto et. al., 2006). Thus, neangiogenesis provides growth and metastasis of tumor.

The study of angiogenesis in practical oncomorphology allowed getting information on the relapses, formation of metastasis, and course of the oncological disease depending on the degree of the development of vessels (Folkman and Klagsburn, 2007). For this purpose CD-31 prognostic factor, which does not depend on the other characteristic features of tumor, is determined in the histological preparation.

Color dopplerographic imaging is considered to be a promising opportunity of ultrasonic examination in the differential diagnostics of the malignant and benign ovarian tumors. Dopplerography allows visualizing tumor blood vessels, including capillaries. High opportunities of the method allow visualizing and identifying even the tiniest blood vessels of the microcirculatory system, which cannot be detected when scanning in the regime B. Twisted, abnormally

shaped vessels more characteristic for malignant tumors can be identified by dopplerography (Ivashina et al., 2012; Fedorova and Lipman, 2012).

Recently, ROC (Receiver Operating Characteristic) analysis has been used for checking the efficiency of the laboratory examination. ROC is considered as a common denominator between the sensitivity and specificity of the examination. ROC allows objectively assessing the diagnostic significance of laboratory tests.

This method can be used for evaluating the tests diagnosing various diseases. ROC allows defining individual criteria for interpreters used in the evaluation of the tests. We can assess the quality of the test according to the deviation of test criterion line from the average line. If the curve of the examined criterion is higher than the average line, then an increase of this parameter has a diagnostic importance and if the curve is lower then a decrease of this parameter has a diagnostic importance.

MATERIALS AND METHODS

Data of the examination of 123 patients diagnosed with malignant ovarian tumors and treated at the Oncological Clinic of the Azerbaijan Medical University during 2008-2015 years have been used in the presented research. The final diagnosis was verified at the laboratory of Patomorphology of Oncological Clinic of AMU. The researches were performed after cytological and histological verification, patients were systematized, the clinical picture of the disease was clarified, and the clinical phases of the process were determined after the summariza-

tion of the results of laboratory instrumental analyses of the patients. According to the research plan all the patients were subjected to ultrasound examination. The examination was carried out through transabdominal and transvaginal sensors at 3.5-5 MHz using the Ultrasound Scanner ALOKA SSD-4000. DAKO ((France) CD 31, Endothelial Cell, clone JC 70A, isotype: Lg GI, kappa 0.2/1ml) reagents were used for histological examinations. Cryostat sections prepared from a material frozen in liquid nitrogen and fixed in acetone and paraffin blocks fixed in a 10% formalin solution were used for the immunohistochemical examination. The results were analyzed using modern statistic methods.

RESULTS AND DISCUSSION

We have revealed that an increase in CD-31 concentration in epithelial ovarian tumors indicates the malignancy. Color doppler examination showed that, the systolic velocity of the blood circulation was higher in malignant tumors compared to the benign tumors and the resistance of the intratumor

blood circulation was lower. Low peripheral vascular resistance in malignant tumors is associated with a sharp increase in the diameter of the arterial vessels. Malignant ovarian tumors are also synthesized by angiogenic factors, which cause the formation of new vessels and further progression of tumors. Histological structure and density of blood vessels are different in diseased and healthy ovary, and this influences on the type of tumor blood circulation.

Using Spirmen's correlation analysis we detected correlation relationship between doplerometric indices of intratumoral blood flow and CD-31 (Table 1). Thus, high expression of CD-31 corresponds to high values of FI. In this case correlation index $r=0.463$, and $p<0.001$ (Figure 1). A strong correlation relationship was observed between VI and CD-31. Correlation coefficient $\rho=0.658$ ($p<0.01$) (Figure 2).

There is also a strong correlation relationship between VFI and CD-31. In this case $\rho=0.463$ ($p<0.01$) (Figure 3).

No relationship was found between PI index and CD 31: $\rho=0.006$ ($p=0.938$) (Figure 4).

Table 1. Correlational relationship between dopplerometric indices of intratumoral blood flow and CD-31 indices

			FI	VFI	RI	PI	CD-31
p-Spearman test	VI	P	0.874**	0.758**	-0.193*	0.121	0.658**
		P	0.000	0.000	0.045	0.210	0.000
	FI	P	1.000	0.861**	-.0273**	0.100	0.463**
		P	.	0.000	0.004	0.300	0.000
	VFI	P	0.861**	1.000	-0.302**	0.157	0.385**
		P	0.000	.	0.001	0.103	0.001
	RI	P	-.273**	-0.302**	1.000	-0.019	-0.051
		P	0.004	0.001	.	0.844	0.675
	PI	P	0.100	0.157	-0.019	1.000	0.006
		P	0.300	0.103	0.844	.	0.958
	CD-31	P	0.463**	0.385**	-0.051	0.006	1.000
		P	0.000	0.001	0.675	0.958	.

Note: statistical confidence of p-correlation coefficient using bivariate criterion; * – $p < 0.05$; ** – $p < 0.01$

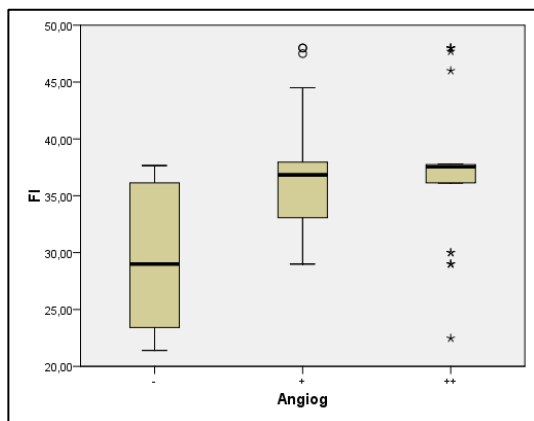


Figure 1. Correlation relationship between FI index and CD-31.

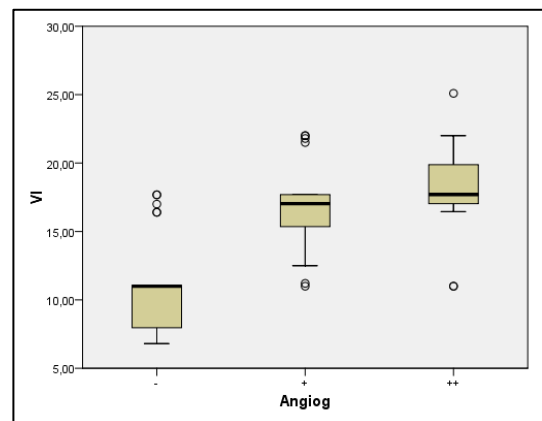


Figure 2. Correlation relationship between VI index and CD-31.

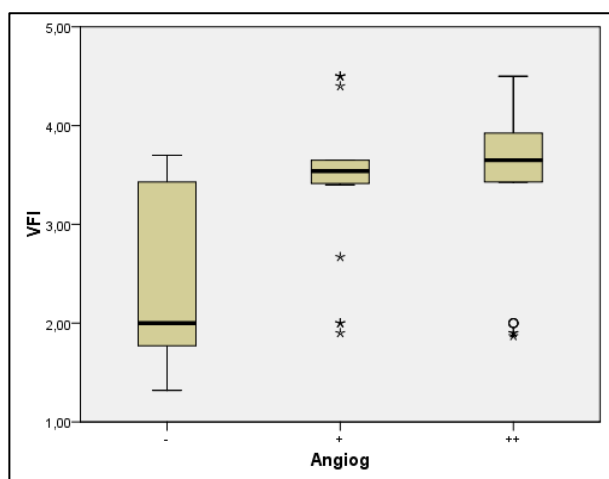


Figure 3. Correlation relationship between VFI index and CD-31.

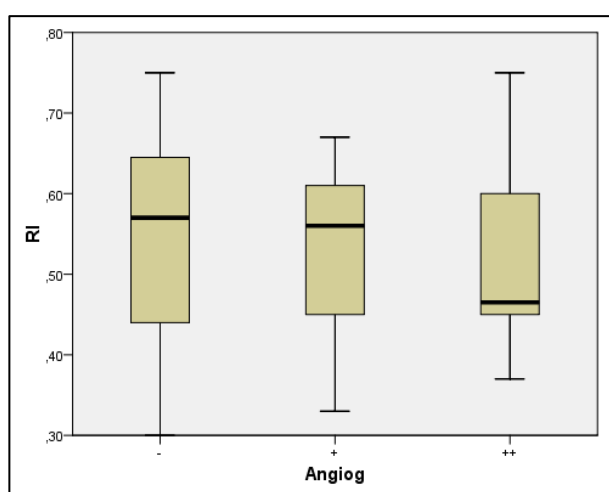


Figure 4. There is no correlation relationship between RI index and CD-31.

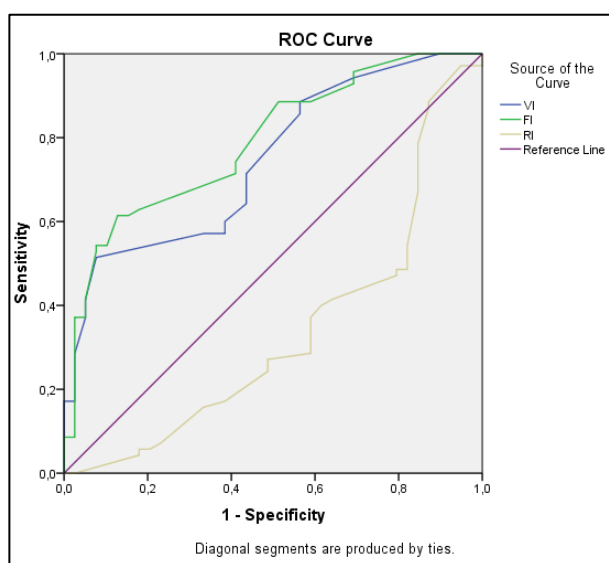


Figure 5. Results of ROS analysis

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig. (p)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Septa thickness	0.748	0.054	0.000	0.642	0.853
VI	0.761	0.056	0.000	0.651	0.872
FI	0.798	0.054	0.000	0.693	0.903
VFI	0.736	0.069	0.001	0.601	0.872
RI	0.374	0.076	0.070	0.225	0.522
PI	0.460	0.076	0.566	0.311	0.609
PSVcm ³	0.641	0.076	0.043	0.492	0.790

Figure 5 shows ROC curves of dopplerometric indices of intratumoral blood flow in malignant ovarian tumors of epithelial origin

ROC curves of dopplerometric indices in malignant epithelial ovarian tumors indicate that dopplerography is of great importance in diagnosis of this disease.

Thus, we have established a positive correlation relationship between dopplerometric indices of blood flow and CD-31. It has been found that increases in dopplerometric indices occur in accordance with CD-31 depending on the degree of malignancy. Thus, high dopplerometric indices of blood flow determine the malignancy of the tumor mass.

Because of the positive correlation between CD-31 and dopplerographic indices, it is possible to determine the characteristics of tumor vasculization using non-invasive method. Thus, the characteristics of tumor can be determined prior to surgery. This is particularly important for the correct selection of treatment tactics and surgery volume. Moreover, based on the doppler examination of malignant ovarian tumors and dopplerometric indices of the blood, the necessity of the administration of antiangiogenic preparations to the patients can be elucidated.

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Bədxassəli Epitel Mənşəli Yumurtalıq Şişlərində Şişdaxili Qan Axınının Doplerometrik Göstəriciləri ilə CD-31 Arasında Korrelyasiyanın Öyrənilməsi

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Bədxassəli yumurtalıq şişində CD-31 ilə doplerometriyanın kəmiyyət göstəriciləri (VI - vaskulyar index, PI – pulsasiya indeksi, RI – rezistentlik indeksi, VFI – vaskulyar axın indeksi) arasında korrelyasiya öyrənilmişdir. Həmçinin bədxassəli yumurtalıq şişlərində doplerometriyanın kəmiyyət göstəricilərinin ROC müayinəsi aparılmışdır. Beləliklə, tədqiqat işinin nəticəsində qanaxının doplerometrik göstəriciləri ilə CD-31 arasında müsbət korrelyasiya əlaqəsi olduğu müəyyən edilmişdir. Məlum olmuşdur ki, şişin bədləşmə dərəcəsindən asılı olaraq CD-31-ə müvafiq olaraq doplerometrik göstəricilərdə də artma baş verir.

Açar sözlər: *Yumurtalıq xərçəngi, CD-31, dopplerografiya, neoangiogenez*

Изучение Корреляции Между Допплеровыми Индексами Внутритрубно́го Кровото́ка и CD-31 При Злокачественных Опухолях Яичников Эпителиального Происхождения

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Изучена корреляция между количественными показателями (индекс сосудистой системы, индекс пульсативности, индекс резистентности, индексы сосудистого потока) доплерометрии и CD-31 при злокачественных опухолях яичников. Также проводилось ROC исследование количественных показателей доплерометрии при злокачественных опухолях яичников. В результате исследования была установлена положительная корреляционная связь между доплерометрическими показателями кровотока и CD-31. Было обнаружено, что увеличение доплерометрических показателей происходит в соответствии с CD-31 в зависимости от степени злокачественности.

Ключевые слова: *Рак яичников, CD-31, доплерография, неангиогенез*

Preliminary Results of Bariatric Surgery in Azerbaijan Population

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Bariatric surgery results in severe obese patients have been reviewed in the Azerbaijani population. The study involved results of the research performed with 119 severely obese patients [average age 40 ± 19 ; average body mass index (BMI) 59.95 ± 20.25 kg/m²] who undergone bariatric surgery, compared post-operative complications by different technical modification, and observed fatty liver disease dynamics by examining BMI, diabetes, hypertension before operation and 1, 3, 6 and 12 months after operation. During the first 6 months, 109 (92.6%) patients who undergone laparoscopic sleeve gastrectomy (LSG) achieved average weight loss of 39.5 ± 11.5 kg. In 10 (8.4%) patients who undergone gastric bypass surgery [2 (1.68%) patients - Ru Y gastric bypass, 6 (5.0%) patients - mini gastric bypass, 1 (0.84%) patient - sleeve + Ru Y gastric bypass and 1 (0.84%) patient - sleeve+mini gastric bypass], this indicator was 46 ± 14 kg, and during the second 6 months effective weight loss was observed as in previous months, which equaled to 33.5 ± 8.5 kg. Annual weight loss indicator was 71.5 ± 23.5 kg for all surgeries. According to our study it can be concluded that unlike other methods, satisfactory weight loss in severely obese patients and consequently, improvement in comorbidities observed after laparoscopic sleeve gastrectomy (LSG) make this method more reliable.

Keywords: Severe obesity, sleeve gastrectomy, gastric bypass

INTRODUCTION

Obesity is a chronic, multisystem disease which causes a number of problems in the human body. This pathology is one of the increasing serious health problems in developed countries. To begin with Type II diabetes and hypertension, as well as impaired venous circulation, hypercoagulopathies, non-alcoholic fatty liver disease, reproductive system disorders are the main complications of this pathology (Sedov et al., 2012). This list can be expanded with orthopaedic complications, arthritis, increase in colon, breast and liver cancer. It has been proved that after surgical correction of this pathology, insulin resistance decreases, and fatty liver disease, hypertension and respiratory pathologies are eliminated by 90% (Yashkov and Ershova, 2011).

Surgical treatment of obesity came up with application and variety of several surgical procedures. Though initial historical information on such operations date back in 1950s, more efficient surgical procedures have begun to be formed since 1979 (Angrisan et al., 2016). However, last breaking point of bariatric-metabolic surgery began in 1992 with adoption of concept on correction of such operations only in surgical way with permanent effects by World Health Organization. Since then, obesity was included in the book of surgical diseases throughout the world, and considered as a surgical pathology. However, it was proposed to use

unique measuring unit, which we now call 'body mass index' (BMI). During the assessment, if BMI is more than 35, surgical treatment, if less, conservation treatment should be applied (Bariatric Today, 2012).

Due to frequent failures, low permanent effects and patients' returning to their previous weight during the treatment of obesity with medicines and dietary methods, the surgical methods have been proven as more efficient procedures. Selection of the best operation type for patient depends on his/her assessed condition on admission and severity of comorbidities (Buchwald and Oien, 2013).

Fundamental concept of bariatric or weight loss surgery, the most efficient treatment of obesity, is to reduce appetite or food absorption from gastrointestinal system, which is implemented by one or both of two key methods: reduce absorption from intestine (malabsorptive procedures) and stomach resection (restrictive procedures).

Obesity surgery is targeted at ideal weight loss of patients, along with helping in treatment of comorbidities. Comorbidities are successfully treated in majority of patients who have undergone surgical procedures against obesity.

Obesity related operations are technically divided into three groups (Felsenreich et al., 2016):

- Operations only restricting nutrient intake (Restrictive methods)
- Operations for low absorption (Malabsorptive methods)

- Operations both for malabsorption and restricting nutrient intake

Our most preferred bariatric surgery method is sleeve gastrectomy.

Laparoscopic sleeve gastrectomy (LSG) or stomach reduction is relatively new surgical method. Average stomach size is reduced by 100-120 ml. Though the key weight loss mechanism of the operation is the reduction of stomach size, Ghrelin hormone produced from the bottom of stomach is not produced after operation, which plays an important role in weight loss and solving metabolic disorders. During the operation, after the bottom of stomach is resected along the major curve by the straight line up to gastro-esophageal junction, this hormone cannot be secreted, which strongly reduces the desire to eat, and consequently, leads to efficient and healthy weight loss. Currently, LSG is the most common type of surgery (Himpens et al., 2010; Hirth et al., 2015).

In laparoscopic Ru-Y gastric bypass, stomach is resected by proximal staple and pouch of approximately 20mm is formed. Jejunum is resected at 50-70 cm distal from ligament of Treitz and connected to stomach pouch with 1cm anastomosis. Proximal part of small intestine is anastomosed to 75-150cm distal part depending on obesity level of patient (Mechanick et al., 2013; Sammour et al., 2010).

In mini gastric bypass or one anastomosis gastric bypass, stomach is resected with proximal staple in parallel with minor curve, and pouch of approximately 60-80mm is formed. Jejunal loop is lifted at 200-250cm distal from ligament of Treitz and connected to stomach pouch with 1cm anastomosis (Stroh et al., 2016).

Objective: Review of bariatric surgery results in severe obese patients in the Azerbaijani population.

MATERIALS AND METHODS

The study involved results of 119 severely obese patients [average age 40 ± 19 ; average body mass index (BMI) $59.95 \pm 20.25 \text{ kg/m}^2$] who undergone open and laparoscopic bariatric surgery during 2013-2016 years.

Surgical instructions are based on 2006 Bariatric Surgery instructions criteria of IFSO (International Federation for the Surgery of Obesity and Metabolic Disorders). Pre-operative weights, BMI and comorbidities of patients were recorded. During the preoperative period, all patients were assessed by gastroscopy for examining upper gastrointestinal system, and by ultrasonography for examining liver, biliary tract pathology. As part of preparation for surgery, all patients passed consul-

tations of pulmonologist, cardiologist, dietician, psychologist and endocrinologist, and an aesthetic risk assessment. Before and after operation heparin fractions were applied, varicose veins socks were dressed up before operation, and dynamical foot masseurs were used. Broad spectrum antibiotic of cephalosporin group was appointed as single dose before operation for prevention, and two doses after operation.

Operations were classified in three groups by their technical modifications. Upon taking standard measures before operation, 109 (92.6%) patients undergone sleeve gastrectomy, 2 (1.68 %) patients - Ru Y gastric bypass, 6 (5.0%) patients - mini gastric bypass, 1 (0.84%) patient - sleeve + Ru Y gastric bypass and 1 (0.84%) patient - sleeve+mini gastric bypass. Operation techniques in all groups complied with international standards. Ru Y gastric bypass and mini gastric bypass were conducted by standard technical methods. However, sleeve gastrectomy was conducted somewhat differently. Though during our review of world literature we observed that 32-42 Fr calibrating tube has no effect on 6-month weight loss, it is advisable to use 32 Fr due to satisfactory long-term results. Moreover, world literature advises to resect at the distance of 4-5cm from pyloric sphincter. During the operations, we complete resection by 32 Fr calibrating tube and 60mm lined staple towards fundus in parallel with minor curve after 2cm distance from pyloric sphincter in antrum. We create smaller stomach as a result of these two methods, and achieve more noticeable and long-term weight loss. In the next stage, methylene blue is injected into stomach, and staple line is controlled. In order to minimize perioperative bleeding and anastomosis leak risk, staple line is sutured (sometimes with omentopexy). In this case, omentopexy is conducted for controlling further gastric torsion, the inner diameter of which is 1cm, and possible leaks from staple line. Drainage is applied to all patients for preventive control of possible staple line leaks. The operation ends with the removal of resected stomach from trocar hole of 15 mm.

Postoperative complications were comparatively investigated in terms of different technical modification, and fatty liver disease dynamics was observed by examining BMI, hypertension before operation and 1, 3, 6 and 12 months after operation.

RESULTS AND DISCUSSION

Out of 119 severely obese patients [average age 40 ± 19 ; average body mass index (BMI) $59.95 \pm 20.25 \text{ kg/m}^2$] included in the study, 98 (82.3%) were females and 21 (17.7%) were males.

Type II diabetes was identified in 34 (28.6%) patients, hypertension in 33 (27.7%) patients, sleep apnoea in 19 (16.0%) patients, polycystic ovarian syndrome-related hormonal dysfunction in 17 (14.3%) female patients, lack of sexual activity in 8 (6.7%) male patients, degenerative osteoarthritis in 14 (11.8%) patients, chronic obstructive lung disease in 3 (2.5%) patient, post-coronary stenting condition related to ischemic heart disease in 2 (1.7%) patients, and Grade 4 fatty liver in almost all patients. Surgical operations were open in two (1.7%) patients, and laparoscopic in remaining (98.3%) patients. Average operation period was 2.5 ± 0.5 hours, and average hospital stay was 2.5 ± 0.5 days. No death was observed. One (0.1%) patient undergone re-operation due to anastomosis leak four days after operation, one (0.1%) patient experiences hypotension the next day after operation and treated with fluid transfer and cardiological medicines. Symptoms of dysphagia were observed three days after operation in one (0.1%) patient, and one month after operation in one (0.1%) patient, and both patients were treated with conservative therapy, and no mechanical tightness was observed during endoscopy. These disorders were normalized after three months without any treatment. Eleven (12.5%) patients undergone abdominoplasty 14 months after operation in order to restore normal appearance.

In 32 patients out of 33 who experienced hypertension before operation, normotensive periods were extended, but in one patient no improvement was observed in hypertension. In 9 patients out of 12 (13.6%) suffering from hypertension, along with fatty liver disease and hyperlipidaemia, these disorders were eliminated within first three months after operation. Despite fasting glucose, disarray in A1C and C-peptide levels during first three months in two patients out of 34 (28.6%) with T2D, fasting blood sugar levels were normalized in the following periods. In one patient, oral dose, as well as combined antidiabetic drug was reduced to a single dose. In majority of male patients (13.6%), deficiency of free plasma testosterone levels and sex hormone-binding globulin levels was identified. These patients demonstrated improvement during six-month control, and by the end of the twelfth month normal ranges were obtained, excluding one patient.

Anastomosis leak from fundal part was observed in one (0.1%) patient five days after LSG. Patient was immediately hospitalized and undergone intensive treatment, as well as drainage of percutaneous liver inferior and left diaphragm inferior. On the next day, fully covered bariatric stent was placed, and the patient was discharged after three days under ambulatory-dynamic control. Fol-

lowing the 5-week dynamic control, the stent was removed, and no complication was observed in subsequent period.

Gastroesophageal reflux was observed in 11 (9.2%) patients two months after operation, dumping syndrome in one (0.1%) patient, who undergone LSG, and diarrhea 5-6 times a day in one (0.1%) patient, who undergone mini gastric bypass. All patients were treated with proton pump inhibitor, acid neutralizers and dietary procedures during the first postoperative 90 days. During the first 6 years, the patients, who undergone standard LSG, achieved weight loss of 39.5 ± 11.5 kg. This figure was 44 ± 13 kg in patients for whom smaller stomach was formed. Weight loss indicator was 46 ± 14 kg in patients who undergone gastric bypass surgery, and during the second 6 months, effective weight loss was observed equaling to 33.5 ± 8.5 kg. However, in patients who undergone standard sleeve gastrectomy, weight loss index decreased in the second 6 months compared to the first 6 months, and equaled to 22.5 ± 4.5 kg. This figure was more efficient and equaled to 28.5 ± 6.5 kg in patients for whom smaller stomach was formed and antrum resection was performed, compared to the standard group. Furthermore, both groups of patients, who were subject to sleeve gastrectomy, did not need any vitamin-mineral support after the first 3 months compared to gastric bypass surgery groups. Patients who had gastric bypass surgery were subject to blood tests once a month, and if needed, received parenteral vitamin-mineral treatment, and were given parenteral vitamin-mineral support under the control of blood tests once in 3 months during 12-month postoperative period. Annual weight loss indicator was 71.5 ± 23.5 kg for all surgeries.

Post-LSG gastroesophageal reflux is worth to be discussed. This issue is caused by loss of cardioesophageal junction during the operation and fast eating without following postoperative diet. We observed this pathology in 11 (12.5%) patients during our study. Some publications of world literature indicated this range as 3-21% (Himpens et al., 2010; Felsenreich et al., 2016). One of the issues reducing the success rate of treatment in obese patients is psychological disorder. Publications investigating the relation between the obesity and psychological disorders cover the symptoms of bipolar disorder in 80% of these patients (Hirth et al., 2015). Although the obesity is not considered as surgical problem, it will be difficult to achieve expected weight loss unless the patients change their old habits of being happy by eating, joy of secret eating and eating too much. Despite that concepts

of successful and unsuccessful bariatric procedure have been recognized until recently, a number of researchers consider 15-50% weight loss resulting from this procedure as successful (Mechanick et al., 2013; Sammour et al., 2010). 42.6% change ratio in excess weight of our patients at the end of twelfth month proves the efficiency of the method. When questioning one patient who lost relatively less weight, we identified regular intake of liquefied chocolate, beer and similar high-calorie drinks during the hunger crisis. This patient returned to normal state after special diet.

Key reason of less weight loss after LSG is insufficient resection. In addition, fistula development, assessment of surgery type, stress and depression, preoperative BMI of more than 80 kg/m² are other factors, which make the treatment of comorbidities difficult.

CONCLUSION

Despite the promising results in short and medium term, long-term results are not sufficient for this method. Unlike other methods, few cases of vitamin deficiency and malabsorption, as well as efficient weight loss ratio after LSG enable wide application of this method. Consequently, LSG is believed to be a reliable method, which ensures sufficient weight loss in the treatment of obesity and super-obesity, as well as improvement in comorbidities.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Statement: Informed consent was obtained from all individual participants included in the study.

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Azərbaycan Populyasiyasında Bariatrik Cərrahiyyə Əməliyyatlarının İlk Nəticələri

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Azərbaycan populyasiyasında aşırı piylənməsi olan xəstələrdə bariatrik cərrahiyyə əməliyyatlarının nəticələri öyrənilmişdir. Tədqiqatda 119 aşırı piylənməsi olan xəstə əhatə edilmişdir [orta yaş 40 ± 19 ; ortalama bədən çəki indeksi (BMI) 59.95 ± 20.25 kq/m²]. Bu xəstələrdə bariatrik cərrahiyyə əməliyyatı aparılmış və əməliyyatdan sonrakı ağırlaşmalar müxtəlif üsullarla müqayisə edilmişdir, təqib dövründə əməliyyatdan sonra və 1, 3, 6 və 12 ay sonra BKİ (bədən-kütlə indeksi), diabet, hipertenziya və yağlanmış qara ciyər göstəricilər müvafiq olaraq yoxlanılmışdır. İlk 6 ay ərzində laparoskopik “sleeve” qastrektomiya keçirən 109 (92.6%) xəstədə ortalama çəki itkisi 39.5 ± 11.5 kq təşkil etmişdir. Mədə şuntlama cərrahiyyəsi keçirən 10 (8.4%) xəstədə [2 (1.68%) xəstədə - “Ru Y gastric bypass”, 6 (5.0%) xəstədə - tək anastomozlu mədə şuntlama əməliyyatı, 1 (0.84%) xəstədə - “sleeve” + “Ru Y gastric bypass” və 1 (0.84%) xəstədə - “sleeve” + “mini gastric bypass”], bu göstərici 46 ± 14 kq təşkil etmişdir, əlavə olaraq əməliyyatdan 6 ay sonra da effektiv çəki itkisi əvvəlki aylarda olan sürətlə davam etmişdir, nəticə 33.5 ± 8.5 kq təşkil etmişdir. Bütün cərrahiyyə növlərində illik çəki itkisi 71.5 ± 23.5 kq təşkil etmişdir. Bizim tədqiqatın nəticələrinə görə, laparoskopik “sleeve” qastrektomiyada (LSG) digər üsullarla müqayisədə ciddi piylənməyə məruz qalmış xəstələrdə müvəffəqiyyətli nəticələr əldə edilmişdir və, müvafiq olaraq, yanaşı xəstəliklərin nəticələri göstəricilərinə müsbət effekt təmin edilmişdir. Bununla biz qeyd edə bilərik ki, laparoskopik “sleeve” gastroektomiya (LSG) digər üsullarla müqayisədə daha əlverişli cərrahiyyə üsulu sayıla bilər.

Açar sözlər: Ciddi piylənmə, “sleeve” gastroektomiya, mədə şuntlama əməliyyatı

Первичные Результаты Бариатрической Хирургии В Азербайджанской Популяции

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Изучены результаты бариатрических операций, проведенных у пациентов с тяжелым ожирением в азербайджанской популяции. В исследование было включено 119 пациентов с тяжелым ожирением [средний возраст 40 ± 19 ; средний индекс массы тела (BMI) 59.95 ± 20.25 кг/м²], которым была проведена бариатрическая операция. У пациентов пост-операционные осложнения сравнивались различными техническими методами, были изучены показатели жирового гепатоза и показатели ИМТ, диабета, гипертонии в динамике перед операцией и 1, 3, 6 и 12 месяцев после операции. В течение первых 6-и месяцев у 109 (92.6%) пациентов, прошедших лапароскопическую рукавную гастрэктомию (LSG) средняя потеря веса составляла 39.5 ± 11.5 кг. У 10 (8.4%) пациентов с операцией шунтирования желудка [2 (1.68%) пациента - «Ru Y gastric bypass», 6 (5.0%) пациентов - «mini gastric bypass», 1 (0.84%) пациент с - «sleeve + Ru Y gastric bypass» и 1 (0.84%) пациент с «sleeve+mini gastric bypass»], этот параметр составлял 46 ± 14 кг, во время операционного ведения в течение 6-и месяцев эффективная потеря веса происходила с той же скоростью, как и в предыдущие месяцы, составив 33.5 ± 8.5 кг. Среднегодовая потеря веса составила 71.5 ± 23.5 кг для всех видов хирургии. В соответствии с результатами нашего исследования мы можем заключить, что в отличие от других методов, эффективная потеря веса и показатели сопутствующих заболеваний при лапароскопической рукавной гастрэктомии делают его наиболее предпочтительным методом лечения тяжелой степени ожирения.

Ключевые слова: Тяжелая степень ожирения, рукавная гастрэктомия, шунтирование желудка

Analysis of the Factors of Longevity by The Data of Survey of the Long-Livers of Lankaran City

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The aim of the study was to substantiate and systematize medico-social approaches to the analysis of gerontological problems. The object of research is long-livers, as a specific socio-demographic group of society. The study included 54 indigenous inhabitants of the city of Lankaran, whose age varied from 85 to 118 years. The conducted study confirmed the importance of behavioral factors of active longevity. From interviews with longevity it becomes clear how much attention during their life was given to them by physical education, diet. The absence of bad habits, coupled with high physical activity, significantly reduces behavioral risk factors in the elderly. But most importantly, what was the most important conclusion that at the heart of longevity, at its very core, lie two important concepts - mutual understanding and love. It is very important to love and be loved throughout life.

Keywords: *long-livers, longevity, analyzes, Lankaran*

INTRODUCTION

According to UN estimates, the world's population aged 60 and over had 600 million people in 2000, almost three times the size of this age group in 1950 (205 million people). In 2009, it exceeded 737 million people, and by 2050 will be more than 2 billion people, once again tripling over a period of 50 years. According to the long-term forecasts of the United Nations, by 2025 the population of the globe will triple in comparison with 1950, and the number of elderly people will increase 6 times, while the number of elderly people over 80 will increase 10 times (Global aging ..., 2012; Shcherbakova, 2012).

The aging process is not unique, it is associated not only with processes of extinction, but also with the emergence of adaptive mechanisms for their suppression and compensation. Various kinds of changes in a person as an individual, occurring in the elderly and old age, are aimed at actualizing the potential, reserve opportunities accumulated in the body during growth, maturity and emerging during late ontogeny (Berdyshev, 1968; Golubev, 2009; World report on aging..., 2015). In old age, not only the activity of some genes is extinguished, but others are stimulated, which ensure a high level of viability of an elderly person. Old age is both an offensive and the possibility of victory over extinction. A healthy lifestyle just helps stimulate offensive genes. A healthy lifestyle, as a system, consists of three main interrelated and interchangeable elements: the culture of nutrition, the culture of

movement and the culture of emotions. It is characteristic that over the past 20 years the number of elderly people aged 85 and older has more than doubled (Berdyshev, 1968; Zhilkina and Dubovitskaya, 2008; Buttner, 2012; Dobrokhleb, 2012).

Countries, meeting at the World Assembly on Aging in 1984 recognized that the quality of life is as important as its longer duration, and therefore aging should, as far as possible, allow living in their own families fruitful, healthy, safe and satisfying life and be considered an organic part of society. In its resolution, the UN World Assembly adopted an action plan on aging and encouraged many countries to develop their national policies and programs for the elders (Tatarinova and Nikitin, 2008; Savchenkov and Sosedova, 2011; Soloviev, 2015).

In this regard, the problem of not just longevity, but active longevity, which is expressed both in the preservation of a satisfactory state of health in old age and in the possibility of prolonging the period of labor activity in old age acquires particular importance (Lee and Mason, 2015; World Population Prospects..., 2015; Piström et al., 2006).

The purpose of the study is:

- substantiation and systematization of medical and social approaches to the analysis of gerontological problems

The attainment of the goal is achieved by solving the following problem, which presupposes:

- analysis of the real life style of long-livers of the city of Lankaran, Azerbaijan under conditions of fundamental transformations of public life;

MATERIALS AND METHODS

The object of research is long-livers, as a specific socio-demographic group of society. The study included 54 indigenous inhabitants of the city of Lankaran, whose age varied from 85 to 118 years.

The subject of the study is the way of life of long-livers.

Modern methods of studying the quality of life.

In our study, we relied on the following methods for determining the quality of life in long-livers:

- 3 approaches to the definition of quality of life:
 1. through subjective well-being;
 2. through social opportunities (capabilities);
 3. through a fair distribution of "objective" resources (social fairness);
- 8 dimensions of complex well-being or well-life:
 1. standard of living (income, consumption, wealth, housing);
 2. health;
 3. education;
 4. personal activity (work, unpaid work, rest, etc.);
 5. political voice, participation, activity;
 6. social relations and relationships;
 7. The environment (in a narrow and broad sense – i.e., including ecology, the state of the environment, current and future);
 8. Insecurity (personal physical and economic security).

The main approaches to the definition of QOL in old age:

- ✓ Objective social indicators;
- ✓ Satisfaction of human needs;
- ✓ Subjective social indicators (based on standard psychological tests);
- ✓ Social capital;
- ✓ Ecology and environment resources;
- ✓ Health and functioning;
- ✓ Psychological models;
- ✓ Hermeneutical approaches.

RESULTS AND DISCUSSION

Almost all interviewed noted that among their relatives were long-livers, but these are mostly grandfathers or grandmothers or uncles and aunts.

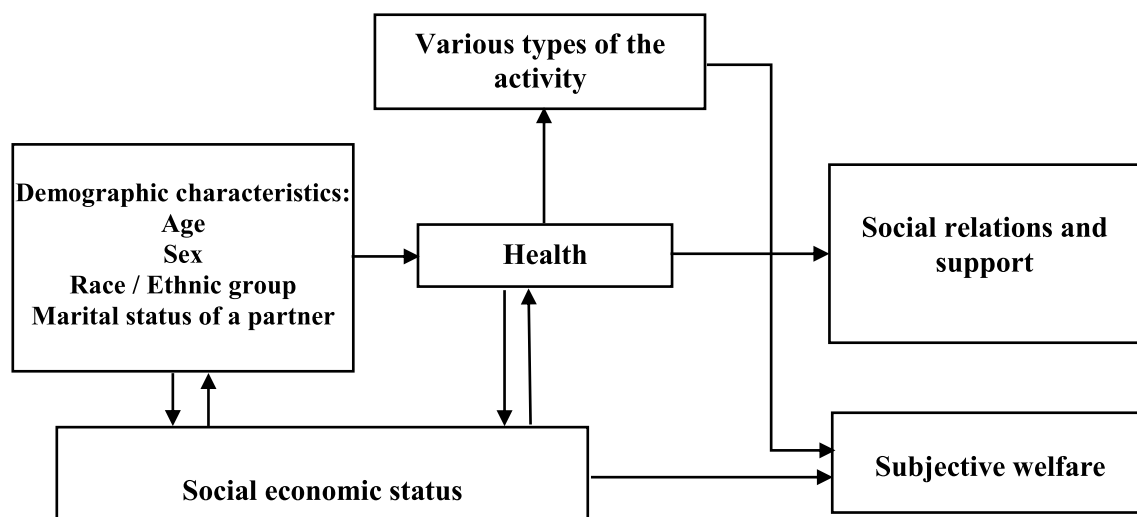
And the overwhelming majority are the women. Direct inheritance of longevity from parents was not revealed.

In the conducted study, we did not reveal the leading role of genetic factors, i.e. it is necessary to study in more detail other factors and, above all, the form and conditions of life.

Long-livers preferred walking all their lives, but, unfortunately, in the last 10 to 12 years, due to sharp changes in the bone system, most have limited motor activity.

The next important element in the formation of the "basis" of longevity is the observance of diet. Features of nutrition in old age are associated with the emerging changes in the digestive system: a decrease in functional activity and atrophy of the glandular epithelium of the stomach, intestines, and liver and pancreas, which together lead to a decrease in the secretion and activity of the produced enzymes.

Conceptual model of the determinants of subjective well-being



The motility of the gastrointestinal tract, digestion and absorption in the intestine are weakened. It is necessary to balance nutrition rationally in accordance with the age, metabolism and energy consumption of the organism. In connection with the decline in assimilating processes in elderly people and the restriction of energy consumption, the need for food in them is reduced to 1900-2200 kcal.

For the elders, four meals a day are recommended, and at a strictly defined time. The first breakfast in 8-9 hours includes 25-30% of the daily ration, the second breakfast at 12-14 hours - 10-15%, lunch at 17-18 hours - 45-50%, dinner at 20-21 hours - 10-20 %. If an elderly person is genetically predisposed to fullness, it is better to eat 5-6 times a day, but little by little, low in calories - at small intervals between meals. Late dinner deprives the rest time of secretory apparatus, which leads to overstrain and exhaustion of the digestive glands. Supper should be easy: a glass of milk, kefir, curdled milk, tomato or fruit juice, fresh fruit, berries. Drinking tea or coffee should not be fulfilled, because they excite the nervous system and disturb sleep.

Sour-milk products have a beneficial effect on the body - stimulate the secretory activity of the stomach, prevent rot in the intestines and normalize peristalsis, well affect the nervous system and metabolism. I.I. Mechnikov believed that one of the causes of aging are the poisons that form in the intestines as a result of the life activity of putrefactive bacteria. Acidic environment for them is unfavorable.

Therefore, the scientist suggested that bacteria of lactic acid fermentation be introduced into the body, which are contained in kefir, curdled milk and similar products.

Basic principles of nutrition in old age:

1. Limitation of consumption of animal fat, contained: in fatty meat, poultry; in dairy products - butter, cream, sour cream; cholesterol-containing products - products, egg yolks, fish eggs. The predominant use of dishes cooked without the addition of fat is in boiled, baked, stewed, or steamed, in a microwave oven, using Teflon-coated dishes.

2. The restriction of added sugar to 30-50 grams, consumed in the composition of various beverages (tea, coffee, compotes, carbonated soft drinks and sour-milk drinks), sweets and confectionery.

3. Restriction of table salt to 5 g per day for cooking, as well as foods high in salt, usually meat and fish delicacies.

4. Enrichment of the diet with polyunsaturated fatty acids. They are contained in vegetable oils (sunflower, olive, linseed, soybean, rapeseed), fatty fish (mackerel, sardines, herring, halibut, catfish, salmon and other kinds of fish from cold seas).

5. The use of sour-milk drinks with low fat content, enriched with useful microorganisms - probiotics.

6. Eating foods rich in dietary fiber. These are raw and boiled vegetables, a variety of fruits and berries, bran and whole wheat bread.

7. The use of foods with high content of magnesium and potassium salts. Among such products - millet, rice, oatmeal, prunes, dried apricots, cabbage, carrots, beets, potatoes, nuts, milk, beef, bran or whole grain bread.

8. The use of products - sources of vitamins C and important for the health of other biologically active substances: broth of wild rose, oranges, sweet red pepper, blueberries, currants, parsley, dill, green onions, gooseberries.

9. Use of foods with high content of B vitamins: bread from wholemeal, bran, legumes; cereals: buckwheat, oatmeal, millet; dairy products, fish.

Meals with relatives and friends, as well as communication with them, also have a positive effect on the well-being and health of the elderly.

Products that need to be consumed regularly in old age: oatmeal and other cereals 1-2 times a day; beans - daily, but at least 3-5 times a week; sour-milk drinks 1-2 times a day; mackerel, herring, sardines, etc. "fatty" fish at least 3 times a week; fruits and berries 1-2 times a day, parsley, dill, coriander, etc. leaf greens - 1-2 times a day; cabbage and other vegetables 1-2 times a day; potatoes - 4-5 times a week; nuts, seeds - every day.

The data obtained during the interviews indicate that almost all the respondents observed the diet in varying degrees throughout their lives. With age, the order of eating was given even more attention. Firstly, many respondents noted that over the course of their life they used mainly natural products, often grown on their own plots. Secondly, it was noted that the most important element of the diet is breakfast, which must necessarily include protein foods (eggs, cottage cheese, milk).

Bad habits. When talking about behavioral risk factors in older age, in addition to low physical activity, alcohol and tobacco consumption are often mentioned. The absence of bad habits is one of the secrets of active longevity. This thesis was confirmed in the course of the research: in the overwhelming majority of respondents said that throughout their lives they did not abuse alcohol and tobacco products. The maximum that some of the respondents could afford was the consumption of alcoholic beverages on holidays and in small quantities.

If in foreign studies part indicates the existence in the life of a long-lover of any hobby, then our subjects allocated only work on their own site.

Effective recovery is unthinkable without achieving mental health. A person with an exhausted nervous system experiences spiritual and physical fatigue. Outstanding modern pathologist G.Selye considered the disease solely as pathological stress or distress. He argued that stress is not what happened to you, but how you perceive it.

All long-livers are people who are well-disposed, big optimists who are able to even see joy in a small one. Smile, laughter transform a person. He will always be welcome in any company, in any society. Laughter is a sign of joy, cheerful mood and mental health.

Along time ago, Doctors included "laughter" in the arsenal of medicines. Laughter favorably affects the work of the lungs, regulates metabolism. Three minutes of laughter is more useful than 15 minutes of gymnastics. V.Shakespeare wrote: "If you did not laugh during the day, it means that you spent that day in vain."

But, it should be noted that optimism is not a natural quality. It is necessary to educate the mood, to be able to control oneself. With retirement, it becomes possible to regulate the rhythm of life. A feasible variety of work with a reasonably organized rest, careful attitude to the nervous system is a pledge of health, a long life.

Stressful situations were helped by strong family and friendly ties. For the respondents we observed, the situation is very typical.

The factor of longevity - both social ties,

Social ties. An active life position promotes the expansion and maintenance of a wide range of social ties. First of all, it should be noted that in the immediate environment of the respondents are usually relatives. Practically in all cases, the families of long-livers include a sufficiently large number of children, grandchildren and great-grandchildren. Wisdom is the basis for fulfilling one of the most important socio-cultural functions of the older generation - the transfer of life experience, and it finds its embodiment mainly in the family circle. In the main body, the subjects lived together with their children. Long-livers play an important unifying role in their families, which, among other things, allows the formation of family traditions.

Labor activity. The most striking feature of the interviewed long-livers is the length of the period of work. All of them started their labor path from a young age.

Standard of living. With regard to the financial situation of the respondents and housing conditions, at the current moment they can be called satisfactory: long-livers do not experience inconveniences in terms of the comfort of living, as well as the acute need for any goods or services. At the same time, the material and housing well-being of the respondents

is explained not only by the socially-oriented nature of state policy aimed at improving the level and quality of life of older citizens, but their residence in the family is of great importance.

Thus, the study confirmed the importance of behavioral factors of active longevity. From interviews with longevity it becomes clear how much attention during their life was given to them by physical education, diet.

The absence of bad habits, coupled with high physical activity, significantly reduces behavioral risk factors of the elders.

But most importantly, what was the most important conclusion that at the heart of longevity, at its very core, lie two important concepts - mutual understanding and love. It is very important to love and be loved throughout life.

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Lənkəran Şəhərində Uzunömürlülülərin Müayinəsinin Nəticələrinə Əsasən Uzunömürlülük Faktorlarının Təhlili

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Tədqiqatın məqsədi herontoloji problematikanın təhlilinə tibbi-sosial yanaşmanı sistemləşdirmək və əsaslandırmaqdan ibarətdir. Tədqiqatın obyektı cəmiyyətin spesifik sosial-demoqrafik qrupu kimi uzunömürlülər olmuşdur. Tədqiqata Lənkəranın yerli əhalisindən olan 54 nəfər daxil edilmişdir. Aparılan söhbətlər məlum olmuşdur ki, onlar tərəfindən bədən tərbiyəsi, qida rejiminə çox böyük əhəmiyyət verilmişdir. Zərərli vərdişlərin olmaması böyük yaş qruplarında davranış faktorlarını aşağı salır. Lakin əsas məsələ odur ki, uzunömürlülüyn əsasını iki məhfum təşkil edir- qarşılıqlı anlaşıma və məhəbbət. bütün ömür boyunca sevmək və sevimli olmaq çox vacibdir.

Açar sözlər: *Uzunömürlülər, uzunömürlülük, müayinələr, Lənkəran*

Анализ Факторов Долголетия По Данным Обследования Долгожителей г. Ленкорани

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Целью исследования явилось обоснование и систематизация медико- социальных подходов к анализу геронтологической проблематики. Объект исследования - долгожители, как специфическая социально - демографическая группа общества. В исследование включены 54 коренных жителя г. Ленкорани, возраст которых варьировал в диапазоне от 85 до 118 лет. Проведенное исследование подтвердило важность поведенческих факторов активного долголетия. Из бесед с долгожителями становится понятно, как много внимания на протяжении жизни уделялось ими занятиям физкультурой, режиму питания. Отсутствие вредных привычек вкупе с высокой физической активностью заметно снижает поведенческие факторы риска в старших возрастах. Но самое главное, что явилось самым важным выводом, что в основе долголетия, в самом ее корне, лежат два важных понятия – взаимопонимание и любовь. Очень важно на протяжении всей жизни любить и быть любимым.

Ключевые слова: *Долгожители, долголетие, анализы, Ленкорань*

Genetic Diversity in Durum Wheat Collections of Azerbaijan Based on SSR Markers

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Genetic diversity and relationships among durum wheat (*T. durum* Desf.) accessions and varieties belonged to 29 botanical varieties was studied using simple sequence repeat (SSR) marker system. A total of 104 alleles were produced using 13 SSR primers for 145 durum wheat accessions, with an average of 8 bands per primer. The mean H_E and PIC values were 0.62 and 0.58, respectively, which indicates high diversity in the studied collection. The higher diversities were obtained for var. *leucurum* (PIC=0.57) and var. *hordeiforme* (PIC=0.51). No clear grouping pattern was revealed based on botanical varieties, with few exceptions, indicating a significant amount of shared alleles among them. The highest similarity was noted between var. *hordeiforme* and var. *melonopus*, while var. *leucurum* and var. *melanopus turgidoid* were the most distant. Rich diversity revealed among durum wheat cultivars and botanical varieties can be used as a valuable source for future breeding programs.

Keywords: Durum wheat, SSR, genetic diversity, botanical varieties

INTRODUCTION

Durum wheat (*Triticum durum* Desf., $2n=4x=28$; AABB) is the only tetraploid wheat, widely used today for human consumption, and the second most widely cultivated wheat specie in the world (Henkrar et al., 2016). It originated in the Fertile Crescent (10,000 BP) and spread over the northern side of the Mediterranean region (Moragues et al., 2006). Durum wheat is characterized by a high polymorphism and for the number of botanical varieties, ecological types and varieties is second only to bread wheat (Flyaksberger, 1938). As a result of the natural and human selection a variety of durum wheat landraces were developed and widely cultivated in many parts of the world until the middle of the 20th century. However, after the Green Revolution they were progressively replaced by the improved, genetically uniform cultivars, which lead to narrowing of their genetic base and loss of diversity. Nevertheless, scientists are convinced that the use of valuable local genetic resources is extremely important and may provide new alleles for the improvement of commercially valuable traits (Soriano et al., 2016).

In Azerbaijan cultivation of wheat has a long history. A wide range of soil and climatic conditions contributed to the development of rich vegetation, this in turn made possible to consider Azerbaijan as one of the most probable centers of wheat origin (Vavilov, 1967). Durum wheat varieties of Azerbaijan - Sary bughda, Gara bughda, Agh bughda, Gyrmizy bughda were mentioned in references since the XIX century (Абдуллаев, 1957). As a

result of breeding activities during 1980s years new durum wheat varieties such as Tartar, Vugar, Barakatli-95 were also created and registered by academician Jalal Aliyev (Əliyev, 1989). In addition, systematic activities have been conducted on collecting and conservation of botanical varieties and populations of different wheat species, as well as *T. durum* widespread in different regions of Azerbaijan. Currently, more than 2,000 wheat accessions are maintained in the National Genebank. These accessions together with durum wheat varieties represent a particularly important group of genetic resources that can be used in breeding programmes in order to increase the available genetic variation, in terms of adaptation to different environments, end-product quality and resistance to diseases.

Knowledge of genetic diversity is crucial for understanding the relationships between cultivars and facilitating their use in new breeding strategies and crosses (Soriano et al., 2016). Molecular markers play a pivotal role in evaluation of genetic diversity (Henkrar et al., 2016). Although several markers have been used for analysis of genetic diversity and variety identification of durum wheat (Chen et al., 1994), microsatellite (SSR) markers proved to be more powerful due to abundance, allele richness, polymorphism and codominance. A number of studies confirmed the usefulness of SSR markers for evaluating the genetic diversity (Royo et al., 2010; Ruiz et al., 2012) and genetic mapping (Yousefi Javan et al., 2011) in different wheat species, as well as in *T. durum*. To date, only few studies have examined the genetic diversity and rela-

tionship among durum wheat accessions of Azerbaijan using RAPD, ISSR and SNP markers (Aliyev et al., 2007; Гаджиев и др., 2015; Abbasov et al., 2017). No work has been focused to study microsatellite diversity in Azerbaijani durum wheat.

The aim of the current study was to evaluate the diversity existing in a durum wheat collection of Azerbaijan using SSR markers and to determine genetic distances among different cultivars and botanical varieties.

MATERIALS AND METHODS

A total of one hundred forty-five accessions of *T. durum* Desf. belonged to 29 botanical varieties were used as a research material. Out of 145 accessions 36 were varieties created in different years.

Genomic DNA isolation was conducted following a CTAB protocol. Thirteen polymorphic SSR markers were used in the study (table 1). The Polymerase Chain Reactions (PCRs) were performed using fluorescent-dye labeled primers as follows: initial denaturation at 95°C for 3 min; 40 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 2 min; final elongation at 72°C for 10 min. Amplified DNA products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific) (Chao et al., 2007). Fragment analysis and allele calling were performed using GeneMapper software v.3.7 (Applied Biosystems).

PowerMarker v. 3.51 (Liu and Muse, 2005) software was used to calculate a total number of alleles, expected heterozygosity (H_e), observed heterozygosity (H_o), polymorphism information content (PIC). Allele frequencies and distances based on frequencies among different botanical varieties were also calculated using PowerMarker. Cluster analysis, PCoA analysis and Neighbour-joining tree was undertaken using software package DARwin 6.0 (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

Exploiting the variability of wheat landraces requires previous knowledge of their genetic diversity.

Genetic diversity and relationships among 145 durum wheat accessions belonged to 29 botanical varieties, including 36 varieties was studied using simple sequence repeat (SSR) marker system. The variation of genetic diversity and allele distribution were strongly dependent on the analyzed loci. A total of 104 amplicons were produced using 13 SSR

primers for 145 durum wheat accessions, with an average of 8 bands per primer (table 1). The number of alleles generated by each primer varied from 2 for *barc182* to 15 for primer pair *barc147*. Ten out of 13 primers produced more than 5 alleles. High number of alleles observed in our experiments can be explained by the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Al-Faifi et al., 2016). Similar observations were also reported by Mangini et al. (2008). The observed heterozygosity was low for the collection ($H_o = 0.07$) and varied from 0.00 to 0.42 with maximum value obtained for primer pair *gwm361*. The low observed heterozygosity level is probably due to the self-pollinated nature of the durum wheat. Major allele frequency, which is inversely proportional to the gene diversity, ranged from 0.21 to 0.93 and averaged 0.51. The main diversity parameters of the locus, the expected heterozygosity (H_e) and the polymorphism information content (PIC) had a wide range. The primer pairs with the maximum value of major allele frequency showed the minimum diversity indices. Among the 13 loci maximum gene diversity ($H_e = 0.87$) and PIC (0.86) values were obtained for locus *barc174*, while loci *barc1021* and *barc182* had minimum H_e and PIC. The majority of the markers, with $PIC < 0.50$, were moderately to highly informative, according to the criteria proposed by Botstein et al. (1980). The mean H_e and PIC values considering all accessions of *T. durum* were 0.62 and 0.58, respectively. The results indicate high diversity in the studied durum wheat collection. The results are higher than the one reported by Carvalho et al. (2009). In his study of 51 durum wheat varieties of Portugal origin, belonging to 26 different botanical species, Carvalho and his colleagues revealed a low level of polymorphism.

Table 1. Summary statistics of the 13 SSR markers used in the study

Marker	Allele No	Major Allele Freq.	H_o	H_e	PIC
<i>barc17</i>	7	0.49	0.03	0.66	0.61
<i>barc212</i>	13	0.35	0.06	0.80	0.77
<i>barc1021</i>	3	0.93	0.00	0.13	0.12
<i>barc117</i>	5	0.54	0.03	0.57	0.49
<i>barc113</i>	8	0.43	0.02	0.68	0.63
<i>gwm332</i>	6	0.37	0.07	0.73	0.68
<i>barc174</i>	8	0.65	0.02	0.54	0.50
<i>barc200</i>	8	0.62	0.19	0.58	0.56
<i>barc147</i>	15	0.21	0.03	0.87	0.86
<i>barc163</i>	11	0.28	0.00	0.82	0.80
<i>barc74</i>	10	0.27	0.07	0.81	0.78
<i>gwm361</i>	8	0.53	0.42	0.67	0.65
<i>barc182</i>	2	0.92	0.00	0.14	0.13
Mean	8	0.51	0.07	0.62	0.58
Total	104				

Table 2. Summary statistics for different subsets of *T. durum* accessions

Subsets	Sample size	Allele No	Major allele frequency	H _O	H _E	PIC
var. <i>affine</i>	7	3.4	0.58	0.03	0.52	0.48
var. <i>africanum</i>	1	1.0	0.88	0.00	0.00	0.11
var. <i>alboobscurum</i>	2	1.5	0.81	0.04	0.20	0.16
var. <i>alboprovinciale</i>	3	2.3	0.58	0.10	0.49	0.40
var. <i>alexandrinum</i>	1	1.2	0.88	0.23	0.12	0.09
var. <i>apulicum</i>	10	2.9	0.67	0.07	0.42	0.38
var. <i>reichenbachii</i>	2	1.3	0.81	0.00	0.00	0.18
var. <i>boeufii</i>	2	1.8	0.62	0.04	0.39	0.30
var. <i>caerulescens</i>	4	2.4	0.60	0.06	0.46	0.39
var. <i>erythromelan</i>	5	3.2	0.56	0.05	0.54	0.49
var. <i>horanomalopus</i>	1	1.2	0.88	0.23	0.12	0.09
var. <i>hordeiforme</i>	15	4.3	0.57	0.08	0.55	0.51
var. <i>leucomelan</i>	9	2.9	0.59	0.08	0.50	0.44
var. <i>leucurum</i>	20	5.0	0.52	0.12	0.61	0.57
var. <i>lybicum</i>	1	1.1	0.96	0.08	0.04	0.03
var. <i>melano leucurum</i>	2	1.7	0.69	0.04	0.32	0.24
var. <i>melanopus</i>	13	3.8	0.59	0.07	0.52	0.48
var. <i>melanopus turgidoid</i>	1	0.9	0.81	0.00	0.00	0.18
var. <i>murciense</i>	6	3.1	0.57	0.04	0.51	0.46
var. <i>mutico affine</i>	1	1.0	1.00	0.00	0.00	0.00
var. <i>mutico africanum</i>	1	1.0	0.88	0.00	0.00	0.11
var. <i>mutico caerulescens</i>	1	0.9	0.92	0.00	0.00	0.08
var. <i>mutico hordeiforme</i>	2	1.6	0.73	0.12	0.28	0.22
var. <i>mutico leucurum</i>	1	0.8	0.65	0.00	0.00	0.34
var. <i>mutico lybicum</i>	1	0.8	0.85	0.00	0.00	0.15
var. <i>muticoobscurum</i>	1	0.8	0.77	0.00	0.00	0.23
var. <i>niloticum</i>	7	2.6	0.70	0.05	0.39	0.35
var. <i>obscurum</i>	8	3.2	0.59	0.09	0.52	0.47
var. <i>reichenbachii</i>	1	0.8	0.85	0.00	0.00	0.15

Among studied botanical varieties, the number of total alleles was associated with the sample size. So, var. *leucurum* and var. *hordeiforme* with the highest sample sizes had the maximum allele numbers as well (5 and 4.3, respectively) (table 2). The higher diversities were obtained for var. *leucurum* (PIC=0.57) and var. *hordeiforme* (PIC=0.51), followed by 5 accessions of var. *erythromelan*. It is interesting to note that, var. *alboprovinciale* and var. *caerulescens* represented by only 3 and 4 genotypes, exhibited higher gene diversity (H_E=0.49 and 0.46). On the contrary, var. *apulicum* with 10 genotypes had relatively lower indices (H_E=0.42; PIC=0.38).

Genetic diversity indices, taking into account the different subgroups (varieties and accessions of different botanical varieties) were little bit lower for varieties (H_E=0.58) in compare with genebank accessions (H_E=0.62). This indicates that the genetic diversity in tetraploid durum wheat was only slightly narrowed down during the second part of the 20th century due to the breeding.

A dissimilarity matrix based on the SSR fragments was used to establish the level of relatedness among durum wheat botanical varieties and varieties. Pair-wise estimates of dissimilarity coefficient (GD) ranged from 0.00 to 1.0 with an average of

0.60. 100% similarity observed for some genotype pairs. Eight main clusters were detected in the dendrogram generated based on SSR dissimilarity matrix (figure 1).

No clear grouping pattern was revealed based on botanical varieties. All clusters were quite diverse containing from 1 to 4 genotypes of several botanical varieties, with some exceptions. This fact indicates the significant amount of shared alleles among different botanical varieties of *T. durum*. Moreover, the studied durum wheat accessions were all collected from one country - Azerbaijan. The relatively narrow collection area (in compare with the germplasm collected from different countries) of samples and thus the similar soil-climatic condition and similar genetic background may also reflect their mixed grouping in the dendrogram. Thus, the low genetic distance estimated between botanical varieties may be explained by geographic proximity. On the other hand, the dissimilarity indices within clusters were also high, indicating high diversity of the studied *T. durum* genotypes.

However, some tendencies were recorded on the grouping of genotypes according to the botanical varieties. Out of 10 genotypes of var. *apulicum* (red colour) 7 fell into cluster VIII.

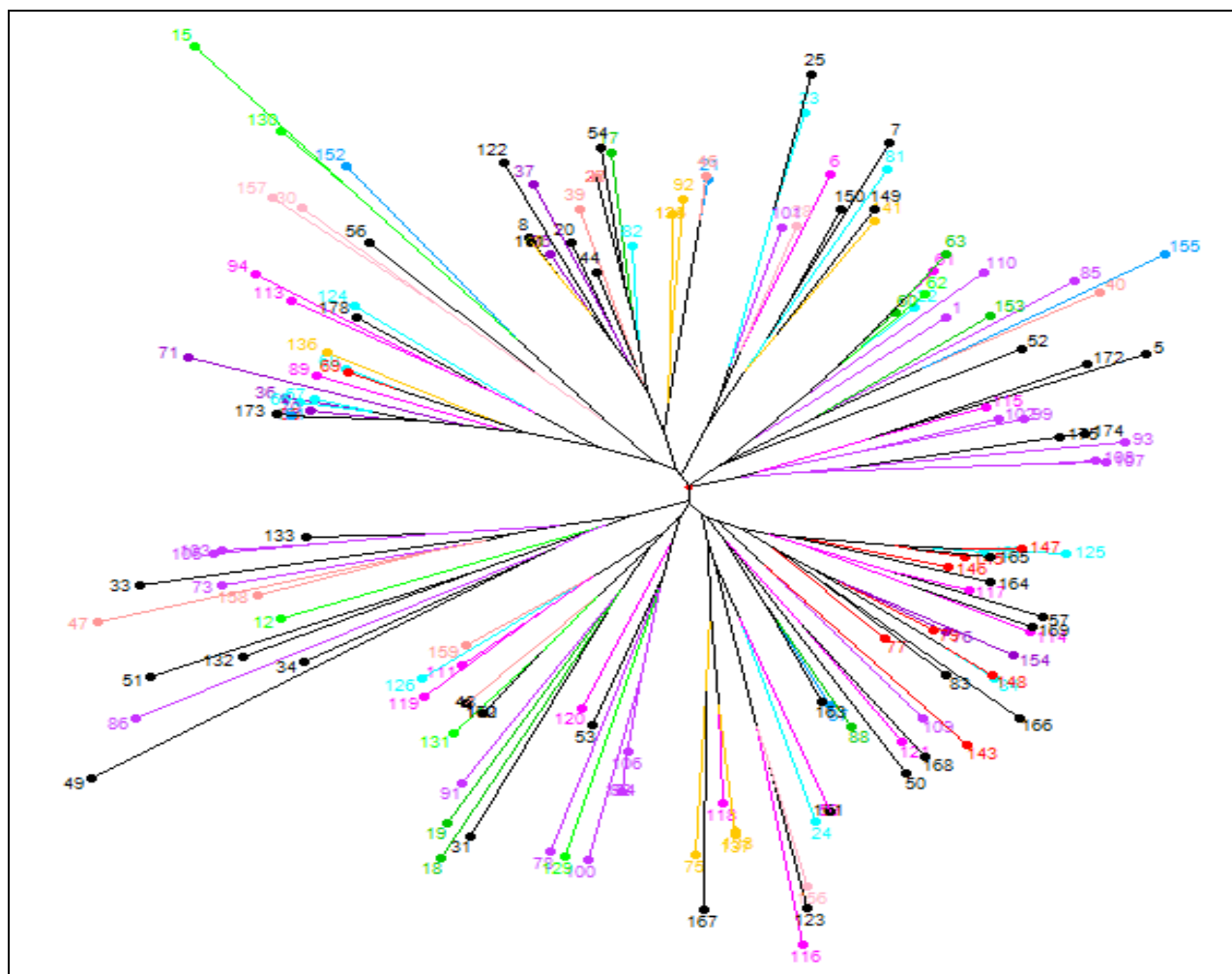


Figure 1. Dendrogram showing genetic relationship among 145 *T. durum* L. accessions based on Nei's genetic distance. Different colors indicate different botanical varieties.

Twenty var. *leucurum* genotypes (violet colour) unequally distributed among clusters; 6 of them were placed very closely in cluster VII, 5 in cluster I and 4 in cluster VI and formed independent groups. Similar grouping pattern was noted for 15 accessions of var. *hordeiforme* (pink colour), 6 of which grouped in cluster VIII and for 9 accessions of var. *leucomelan* (yellow colour); 4 of them were in cluster IV and 3 in cluster VIII with 100% similarity between some pairs. The most diverse cluster in terms of botanical varieties was clusters II and III.

Nei's genetic distance between varieties and genebank accessions was very low ($GD=0.062$). In a number of studies durum wheat landraces are considered as a source of variability that able to provide new favorable alleles to be introgressed into modern cultivars (Lopez et al., 2015). The low genetic distance between varieties and landraces in the current study shows the effective use of durum landraces by durum wheat breeding programmes.

Among studied 36 improved cultivars Sharg and Jafari had same alleles for all 13 SSR loci and thus showed 100% similarity. This was in accordance with Abbasov et al. (2017). The results of the cluster analysis based on 1058 SNPs obtained from GBS analysis confirmed the high similarity between Sharg and Jafari (Abbasov et al. 2017).

In addition, Mugan variety exhibited 100% similarity with genotype from var. *leucomelan*, Savalan with var. *erythromelan* and Barakatly 95 with var. *hordeiforme*. Varieties Vugar and Shiraslan 23 were closer to each other, as well.

PCoA analysis confirmed subgrouping obtained by cluster analysis and showed intermixing of studied botanical varieties across the coordinates (figure 2). The first five axes explained 29.4 % of cumulative variation.

All genotypes were distributed along the scatter plot and did not form genetically well differentiated groups.

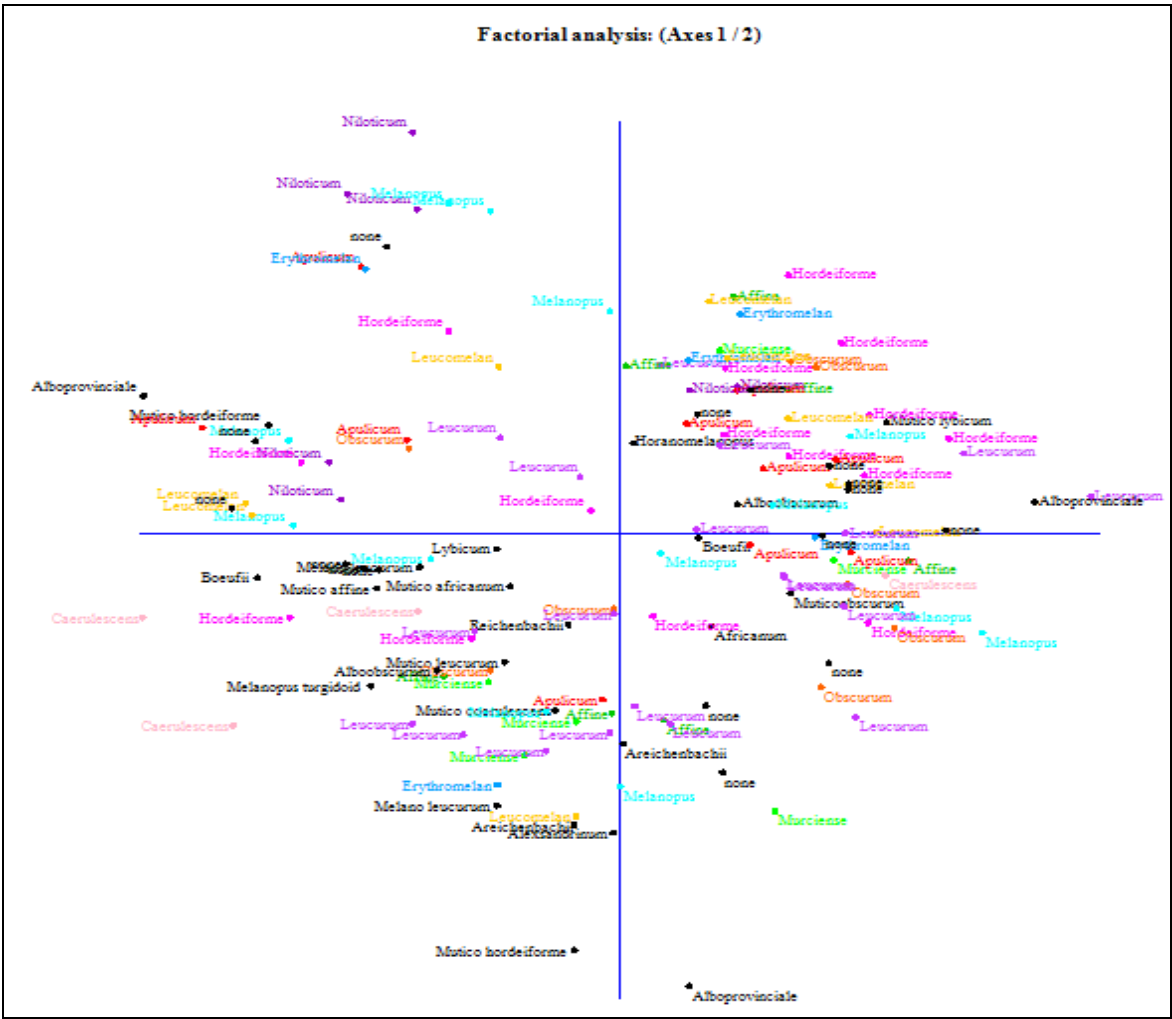


Figure 2. Scatter plot of 145 *T. durum* accessions using 13 SSR markers. Different colors indicate different botanical varieties.

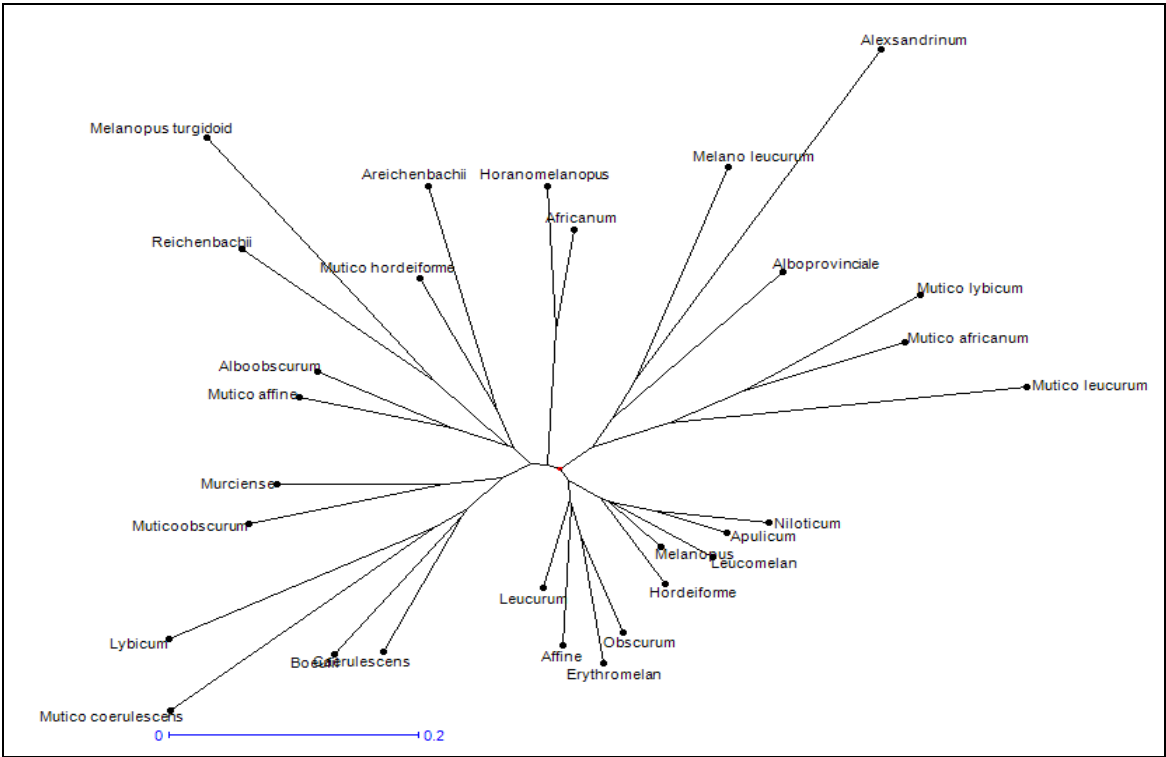


Figure 3. Dendrogram of *T. durum* botanical varieties based on Nei's genetic distance.

Genetic dissimilarity (GD) index among different botanical varieties ranged from 0.13 to 0.86, with mean value of 0.47. The highest similarity was noted between var. *hordeiforme* and var. *melonopus*, while var. *leucurum* and var. *melanopus turgidoid* were the most distant. Similarly, Hajiyeve et al. (2015) recorded close relationship among mentioned botanical varieties. In another study of durum wheat accessions belonged to 13 botanical varieties Abbasov and his colleagues revealed high similarity within var. *leucurum*, followed by var. *hordeiforme* and var. *leucomelan*; they formed separate groups with minimal distances among them (Abbasov et al., 2017).

To visually demonstrate a dendrogram was created based on allele frequencies where 3 main clusters were further divided into several subclusters (figure 3). Var. *leucurum*, var. *affine*, var. *erythromelan* and var. *obscurum* formed a separate subcluster within cluster I, indicating their similar genetic background. The second subcluster contained closely located botanical varieties var. *hordeiforme*, var. *melanopus*, var. *leucomelan*, var. *apulicum* and var. *niloticum* with dissimilarity indices between 0.13 and 0.20.

Among the rest botanical varieties var. *carulescens* and var. *boefferi* (GD=0.29); var. *murciense* and var. *muticoobscurum* (GD=0.29); var. *alboobscurum* and var. *muticoaffine* (GD=0.24) were closer to each other.

So, the present study shows that SSR markers can be used effectively to estimate genetic diversity and relationship among durum wheat genotypes. The SSR technique confirmed the existence of rich diversity in *T. durum* cultivars and botanical varieties of Azerbaijan origin. The studied collection of 145 durum wheat varieties has rich genetic diversity, which is reflected in high values of allele number, expected heterozygosity and PIC. Along with that, they share alleles among themselves, which make impossible their clear differentiating to the clusters. The valuable diversity revealed in the current collection can be used as a source for future breeding programmes.

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SSR Markerlərlə Azərbaycan Mənşəli Bərk Buğda Kolleksiyasında Genetik Müxtəlifliyin Qiymətləndirilməsi

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29 növmüxtəlifliyinə aid bərk buğda (*T. durum* Desf.) sort və nümunələrində genetik müxtəliflik və genetik əlaqə SSR markerləri ilə tədqiq edilmişdir. Ümumilikdə, 13 SSR praymeri ilə 145 bərk buğda nümunəsi üçün 104 allel sintez olunmuş, hər praymerə düşən allel sayı 8 ədəd təşkil etmişdir. Gözlənilən heteroziqotluq (H_E) və polimorfizm informasiya tutumu (PIC) parametrlərinin orta göstəricisi, müvafiq olaraq, 0.62 və 0.58 vahid təşkil etmişdir ki, bu da kolleksiyada yüksək genetik müxtəlifliyin olduğunu göstərir. Növmüxtəliflikləri arasında ən çox müxtəliflik var. *leucurum* (PIC=0.57) və var. *hordeiforme* (PIC=0.51) nümunələri üçün qeydə alınmışdır. Dendrogramda bəzi istisnalar olmaqla, növmüxtəlifliklərinin aydın şəkildə qruplaşması müşahidə olunmamış, növmüxtəlifliklərinin əhəmiyyətli dərəcədə ortaq allellərə malik olduğu müəyyən edilmişdir. Var. *hordeiforme* və var. *melonopus* növmüxtəlifliklərinin bir-birinə ən yaxın, var. *leucurum* və var. *melanopus turgidoid* növmüxtəlifliklərinin isə ən uzaq olduğu aşkar edilmişdir. Bərk buğda kolleksiyasında aşkar edilmiş zəngin müxtəliflik qiymətli mənbə kimi gələcək seleksiya işlərində istifadə oluna bilər.

Açar sözlər: Bərk buğda, SSR, genetik müxtəliflik, növmüxtəlifliyi

Изучение Генетическое Разнообразие Коллекции Азербайджанской Твердой Пшеницы на Основе SSR Маркеров

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С помощью SSR маркеров изучено генетическое разнообразие и генетическая связь между сортами и образцами твердой пшеницы (*T. durum* Desf.), принадлежащими к 29 разновидностям. В общей сложности, для 145 образцов твердой пшеницы с применением 13 SSR праймеров были синтезированы 104 аллели при среднем значении 8 аллелей на локус. Средние значения ожидаемой (H_0) гетерозиготности и величины информационного полиморфизма (PIC) составили 0,62 и 0,58 соответственно, что указывает на большое разнообразие в изученной коллекции. Наибольшее генетическое разнообразие было выявлено для разновидностей var. *leucurum* (PIC=0,57) и var. *hordeiforme* (PIC = 0,51). За некоторыми исключениями, на дендрограмме не обнаружено четкой группировки разновидностей и установлено, что разновидности обладают значительным количеством общих аллелей. Наибольшее генетическое сходство отмечено между разновидностями var. *hordeiforme* и var. *melanopus*, в то время как var. *leucurum* и var. *melanopus turgidoid* оказались самыми отдаленными. Выявленное среди коллекции твердой пшеницы богатое генетическое разнообразие может быть использовано в качестве ценного источника для будущих селекционных программ.

Ключевые слова: Твердая пшеница, SSR, генетическое разнообразие, разновидности

Classification and Ecobotanical Study of Crop Wild Relatives: A Case-Study in Azerbaijan

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For the first time crop wild relatives (CWR) have been classified according to international classification in Azerbaijan. It has been established that there are 965 ancestor species belonging to the 122 genera and 34 families, related to 5 gene groups. As a whole, there are 124 principal crop wild relatives which belong to I and II groups. Others are also important as a genetic resources and have various valuable properties.

Keywords: *Crop wild relatives, gene groups, ecobotany, taxonomy, Azerbaijan*

INTRODUCTION

One of the important directions of the research of plant genetic resources is to study wild ancestors (progenitor) of cultivated plants with food and agricultural importance, their effective use and protection (Heywood et al, 2007). Food, fodder, technical, medicines, vitamins and other important plant species have begun to be cultivated since ancient times for their use by people. The term wild relatives of cultivated plants (WRCP) or crop wild relatives (CWR) are not a precise one and have been variously defined and debated (Heywood et al, 2008). Species (subspecies, variety, etc.) considered CWR are close to that cultural plant species in evolutionary and genetic terms and they are plants widespread in natural flora and suitable for passing into the culture or obtaining new species including the same species. The following issues always make specialists creating new plant species to think about them: species should be resistant to stress factors; it should be resistant to diseases and pests and give higher quality products. In this field, main success of breeders depends on the use of more in situ materials. Therefore, at present, it is very important to study, keep of CWR and detect new ones. In recent years, increase in the role of global warming, technologic processes and other anthropogenic factors negatively affects biota, as well as, CWR; minimizes their aerals and leads to their destruction. Anthropogenic changes are undoubtedly increasing the rates of specific and genetic extinction (Maxted, 2003). Accelerating rates of species extinctions were identified at that time as threats to the genetic base of world agriculture and effort and resources were expended during the following decades to collect CWR and maintain them in ex situ conservation programs (Meilleur and Hodgkin, 2004). Although more than half of the world's flora of higher plants is useful for food and

agriculture, only 2.8 % of them were passed into the state of cultural planting (Zhukovsky, 1964). Only 5 % of 4961 higher plant species in 1117 genera existing in natural flora is observed in the cultural form in Azerbaijan (Asgarov, 2016). It is clear from these examples that although our natural flora comprises rich gene pool of important agricultural plants, this richness is still weakly used. In other words, wild relatives of cultural plants have been studied in situ. For example, though 156 species of *Astragalus* species are widespread in Azerbaijan's flora, examples of seed belonging to only 5 species have been collected till now (Asgarov, 1991). Intraspecific diversity and populations of many species widespread in Azerbaijan's flora are poorly studied. Study of cultural plants with a scientific basis, and determination of the sources of their formation is related to the name of the Russian scientist, N.Vavilov (Vavilov, 1967). Later, since 1935, his multivolume "Cultural Flora of the USSR" works began to be published in Moscow and St. Petersburg. "Cultivated plants and their relatives" by P. Zhukovski (Zhukovsky, 1964), "Wild relatives of cultivated plants of flora of the USSR" by D. Brezhnev and others (Brezhnev et al., 1980), as well as, multivolume "Flora of Azerbaijan" and 3 volumes "Higher plants of Azerbaijan", "Review of the flora of Azerbaijan", "The world of plants of Azerbaijan", by Asgarov have been used (Asgarov, 2005, 2006, 2011, 2016). "Summary of the flora of Caucasus" of which 4 volumes are printed yet is of great importance in the investigation of habitats of CWR and taxonomy issues (Taktajan, 2012). There are valuable research works of I.D.Mustafayev, J.A.Aliyev, K.Abdullayev, A.Rajabli on CWR in Azerbaijan. At present, these works are conducted at the Institute of Genetic Resources of Azerbaijan National Academy of Sciences (ANAS) in a systematic manner.

The main aim of the studies was to implement valid inventarisation of CWR in Azerbaijan, develop their modern classification, identify effective ways of use of them and organize the protection of genomes.

MATERIALS AND METHODS

The habitat of species, their planting conditions is specified by expeditions organized on different routes; herbarium and seed materials on them are collected, information about their planting conditions, as well as, GPS information is recorded. Special attention is paid to the documentation of plant seeds on descriptors. Special rules and procedures are followed during the collection of herbarium and seed materials on rare, endangered, and relic species. Seed and germplasm materials are collected for the purposes of their reintroduction.

Various methods are used on the study of wild relatives of cultural plants: comparative morphological, taxonomic, floristic, geobotanical, bioecological, hybridological and others. Improvement of study methods of CWR is considered to be one of the main duties. Especially, among these, there are compiling the electronic maps of habitats of CWR, study of useful features, molecular-genetic nature of ontogenesis etc.

International Treaty on Plant Genetic Resources adopted by the UN Food and Agriculture Organization (FAO) (2017), the Harlan and de Wet inventory (Holly et. al., 2013), information of Central Database of Genetic Resources Institute has been used in the identification of priority species of wild relatives of cultural plants. At that time, first and second centers were determined. Usually, more ancient and primitive forms are gathered in the first center, and young forms with intense diversity are concentrated in the second center.

Another method is systematic method. The essence of this method lays in the fact that progenitor,

as a rule, is located in the same semi-species, section or series with the closest wild species in phylogenetic terms in systematic hierarchy.

RESULTS AND DISCUSSION

965 species of CWR belonging to 122 genera on 34 families were found in the flora of Azerbaijan (except decorative plants) have been identified and it has determined that they belong to 5 groups of gene pool (Table 1). Azerbaijan can be considered one of the richest regions in Transcaucasian group of formation source of cultural plants in Southwest Asia. Here, among very valuable ancestor species, there are cereal grains (wheat, barley, oats, wheatgrass, rye, etc.), grain legumes (heath pea, peas, etc.), fruits (apples, almonds, grapes, medlar, walnuts, etc.), wild vegetables (onions, beets, carrots, strawberries, etc.), among feed plants: legumes fodder plants (trigonella, shamrock clover, alfalfa, melilot, sainfoin etc.), forage grasses (couch-grass, poatrivialis etc.) numerous oil, spice, herbal, vitamin plants can be mentioned. There are 124 principal crop wild relatives in Azerbaijan which were included gene pool 1 and 2 (Table 2). Some CWR were included in the list which crops were not cultivated in Azerbaijan such as *Eleusine coracana* and *Chenopodium quinoa*. Azerbaijan was considered formation centers of many of these plants. For example, among some important wild relatives of plant all over the world, wheat types (*Triticum boeoticum*, *T.spontaneum*, *T.urartu*, *T.araraticum* and others), their diversity of species more than a hundred, barley varieties (*Hordeum bulbosum*, *H.geniculatum*, *H.violaceum* and other) types of wheat grass (*Aegilops kotschy*, *A.tauschii*, *A.umbellulata* and others), rye types (*Secale vavilovii*, *S.anatolicum*, *S.segetale* and others), in addition, many feed (*Medicago*, *Onobrychis*, *Vicia*, *Picum* and others), fruit, vegetables and melons etc. can be shown.

Table 1. Prioritization concepts used in the creation of the Azerbaijan crop wild relative (CWR) list.

Prioritization concept	Sublevel description	Number of taxa in Azerbaijan
GP1	Wild species being in culture and represented by breeding varieties.	42
GP2	Prospective species for using in agriculture being phylogenetically close to the type of species passed into culture (including in the same semi-species, same selection and series).	82
GP3	Species used as a source of gene or being useful as grafting means in hybridization activities.	175
GP4	Species used in breeding and having useful properties (there is no any official breeding species and has not been passed into culture).	243
GP5	All other types of species having the type represented with breeding species and being in culture: species whose useful features and usage in the economy is less studied, as well as, being rare and endangered, advent and not fully naturalized, but degenerating and growing wild.	419

Table 2. List of global priority crop wild relatives of Azerbaijan

Crop name	Species name	Crop name	Species name
Cereals			
Wheats	<i>Triticum timopheevii</i> (Zhuk.) Zhuk. <i>T. urartu</i> Thum. ex Gandil. <i>Aegilops biuncialis</i> Vis. <i>A. columnaris</i> Zhuk. <i>A. crassa</i> Boiss <i>A. cylindrica</i> Host <i>A. geniculata</i> Roth <i>A. kotschy</i> Boiss. <i>A. neglecta</i> Reg. ex Bertol <i>A. tauschii</i> subsp. <i>tauschii</i> Coss. <i>A. triuncialis</i> L.	Sweet cherry	<i>P. syriaca</i> Boiss. <i>Prunus avium</i> (L.) L. <i>P. cerasus</i> L.
		Apricot	<i>P. × dasycarpa</i> Ehrh. <i>P. armeniaca</i> L.
		Myrobalan plum	<i>P. cerasifera</i> Ehrh.
		Plum	<i>P. domestica</i> L. <i>P. spinosa</i> L.
		Sour cherry	<i>P. mahaleb</i> L. <i>P. padus</i> L.
		Peach	<i>P. persica</i> (L.) Batsch
		Strawberry	<i>Fragaria × ananassa</i> (Duchesne ex Weston) Duchesne ex Rozier
		Fig	<i>Ficus carica</i> L.
Crest. wheatgrass	<i>A. umbellata</i> Zhuk. <i>Agropyron cristatum</i> (L.) Gaertn. <i>A. desertorum</i> (Fisch. ex Link) Schult. <i>A. fragile</i> (Roth) P. Candargy	Blackcurrant	<i>Ribes petraeum</i> Wulfen <i>R. uva-crispa</i> L.
Oat	<i>Avena barbata</i> Pott ex Link <i>A. eriantha</i> Durieu. <i>A. fatua</i> L. <i>A. sterilis</i> L. <i>A. ventricosa</i> Balansa	Almond	<i>Prunus dulcis</i> (Mill.) D.A. Webb <i>P. fenzliana</i> Fritsch
Finger millet	<i>Eleusine indica</i> (L.) Gaertn. <i>E. tristachya</i> (Lam.) Lam.	Legumes	
Int. wheatgrass	<i>Elymus elongates</i> (Host) Runemark <i>E. hispidus</i> (Opiz) Melderis	Sweetpea	<i>Lathyrus annuus</i> L. <i>L. chloranthus</i> Boiss. <i>L. cicera</i> L. <i>L. hirsutus</i> L. <i>L. sylvestris</i> L. <i>L. tuberosus</i> L.
Barley	<i>Hordeum brevisubulatum</i> (Trin.) Link <i>H. bulbosum</i> L. <i>H. spontaneum</i> K. Koch <i>Panicum miliaceum</i> L.	Lentil	<i>Lens culinaris</i> subsp. <i>orientalis</i> (Boiss.) Ponert <i>L. ervoides</i> (Brign.) Grande <i>Medicago littoralis</i> Loisel. <i>M. papillosa</i> Boiss. <i>M. rigidula</i> (L.) All. <i>M. sativa</i> subsp. <i>varia</i> (Martyn) Ar-cang.
Broom millet	<i>Pennisetum orientale</i> Rich.	Alfalfa	<i>M. truncatula</i> Gaertn. <i>Pisum sativum</i> subsp. <i>elatius</i> (M. Bieb.) Asch. & Graebn. <i>Vicia ciliatula</i> Lipsky <i>V. ervilia</i> (L.) Willd. <i>V. grandiflora</i> Scop. <i>V. hybrida</i> L. <i>V. hyrcanica</i> Fisch. & C.A. Mey. <i>V. johannis</i> Tamamsch. <i>V. lutea</i> L. <i>V. narbonensis</i> L. <i>V. pannonica</i> Crantz <i>V. sativa</i> subsp. <i>amphicarpa</i> (Dorthe) Asch. <i>V. sativa</i> subsp. <i>nigra</i> (L.) Ehrh. <i>V. serratifolia</i> Jacq. <i>C. murale</i> L. <i>C. opulifolium</i> Schrad. ex W.D.J. Koch & Ziz <i>C. polyspermum</i> L. <i>C. strictum</i> Roth <i>C. urbicum</i> L. <i>C. vulvaria</i> L.
Pearl millet		Pea	<i>Spinacia tetrandra</i> Steven ex M. Bieb.
Rye	<i>Secale segetale</i> (Zhuk.) Roshev. <i>S. sylvestre</i> Host.	Vetch	<i>Carthamus glaucus</i> M. Bieb. <i>C. gypsicola</i> Iljin <i>C. lanatus</i> L. <i>C. oxyacantha</i> M. Bieb. <i>Solanum sisymbriifolium</i> Lam.
Foxtail millet	<i>S. vavilovii</i> Grossh. <i>Setaria italica</i> (L.) P. Beauv. <i>S. verticillata</i> (L.) P. Beauv. <i>S. viridis</i> (L.) P. Beauv. <i>Sorghum halepense</i> (L.) Pers.		
Sorghum			
Fruit crops			
Apple	<i>Malus orientalis</i> Uglitzk. ex Juz.		
Pear	<i>Cydonia oblonga</i> Mill. <i>Pyrus boissieriana</i> Buhse <i>P. caucasica</i> Fed. <i>P. pyrifolia</i> (Burm. f.) Nakai <i>P. salicifolia</i> Pall. <i>Allium ampeloprasum</i> L. <i>A. atroviolaceum</i> Boiss.		
Garlic			
	<i>A. saxatile</i> M. Bieb. <i>A. scabriscapum</i> Boiss. <i>A. schoenoprasum</i> L. <i>Beta lomatogona</i> Fisch. & C.A. Mey. <i>B. macrorhiza</i> Steven	Spinach	
Sugarbeet		Safflower	
Carrot	<i>B. vulgaris</i> subsp. <i>maritima</i> <i>Daucus carota</i> L.		
Asparagus	<i>Asparagus officinalis</i> L. <i>A. verticillatus</i> L.		
Lettuce	<i>Lactuca azerbaijanica</i> Rech. f. <i>L. georgica</i> Grossh. <i>L. saligna</i> L. <i>L. serriola</i> L.	Aubergine	
		Nut crops	
		Hazelnut	<i>C. avellana</i> L. <i>C. columnata</i> L.

Table 2 continued

Crop name	Species name	Crop name	Species name
Vegetables		Quinoa	<i>Chenopodium album</i> L.
Rape	<i>Brassica elongata</i> Ehrh.	Walnut	<i>Juglans regia</i> L.
	<i>B. nigra</i> (L.) K. Koch	Pistachio	<i>Pistacia atlantica</i> Desf.
	<i>B. oleracea</i> L.		
	<i>B. rapa</i> L.		
	<i>B. tournefortii</i> Gouan		
Radish	<i>Raphanus raphanistrum</i> L.		

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Azərbaycanda Mədəni Bitkilərin Yabani Əcdadlarının Təsnifatı və Ekobotaniki Tədqiqi

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Məqalədə ilk dəfə olaraq Azərbaycanda yayılan mədəni bitkilərin yabani əcdadları (sələfləri) Beynəlxalq təsnifata uyğun olaraq təhlil edilir. Müəyyən edilmişdir ki, Azərbaycanda 34 fəsilə, 122 cinsə aid 965 növ sələf bitkiləri 5 gen qrupuna aiddir. Onlardan 124 növ bilavasitə sələf bitkisi olmaqla prioritet olan I və II qruplara aiddirlər və seleksiya işlərində geniş istifadə oluna bilərlər. Digər növlər genetik ehtiyat kimi qiymətlidirlər. Müxtəlif faydalı xüsusiyyətlərə malikdirlər.

Açar sözlər: Mədəni bitkilərin yabani əcdadları, gen qrupu, ekobotanika, əcdad, Azərbaycan

Классификация и Экоботанические Исследования Диких Сородичей Культурных Растений Азербайджана

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В статье приводится таксономический состав диких сородичей культурных растений Азербайджана в соответствии с Международной классификацией. Установлено, что в Азербайджане 965 видов диких сородичей, относящихся 122 родам и 34 семействам, в общей сложности относятся к 5 генетическим группам. Из них 124 вида являются приоритетными и относятся к I и II группам. Они могут быть широко использованы в селекционных работах. Другие виды также являются ценными генетическими ресурсами и обладают различными полезными свойствами.

Ключевые слова: *Дикие сородичи культурных растений, генгруппы, экоботаника, вид, род, Азербайджан*

Evaluation of Biomorphological Diversity and Distribution of *Lathyrus* L. s. l. Species in Azerbaijan

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As a result of monitoring conducted under natural conditions, 13 seed samples and 80 herbarium specimens, belonging to 9 species of genus *Lathyrus* L. were collected in Azerbaijan in 2016. The article presents the results of the research on biomorphological diversity and dissemination based on their descriptor data.

Keywords: *Lathyrus* L., species, genus, Azerbaijan, ecological-botanical

INTRODUCTION

Lathyrus L. is one of the polymorphic varieties of legumes. *Lathyrus* L. species are valuable feed, medicinal and decorative plants. It contains lots of proteins and other biologically active ingredients, and its dry grass is well eaten. Sweet pea (*L. odoratus*) is a widely used ornamental plant (Sarker et al., 1997). Some species are melliferous. Meadow vetchling (*L. pratensis*) is a herbal plant (Grossheim, 1952; Karyagin, 1954).

Researchers divide 170 species of *Lathyrus* L. into (Kenicer, 2008; Lewis, 2005) 12 to 13 sections throughout the world (Chefranova, 1971, 1987; Asmussen et al, 1998; ILDIS, 2010; Kupicha, 1974, 1983; Leht, 2009). There are 24 species as wild with *Orobanchaceae* L. in Azerbaijan (Asgarov, 2011, 2016; Karyagin, 1954). One species is cultivated (*L. odoratus*). Five of the *Lathyrus* species spread in Azerbaijan were referred to as *Orobanchaceae* L. (Grossheim, 1952; Karyagin, 1954), which is currently not regarded as an independent species and is included in the composition of the *Lathyrus* species (Asgarov, 2011, 2016; Magulaev, 2005). Unlike the *Lathyrus* species, in *Orobanchaceae* L. species, the leaves are without cirrus, and instead of it, there is a protrusion at the end of the leaf. This sign is now regarded as the main characteristic of the *Lathyrus* species referred to the *Orobanchaceae* section (Asgarov, 2011, 2016; Grossheim, 1952; Fedchenko, 1948; Chefranova, 1971, 1987; Asmussen et Liston, 1998; Bässler, 1973).

Though A. Grossheim (Grossheim, 1952) gave information about the types of *Lathyrus* spread throughout the Caucasus and I. Karyagin (Karyagin, 1954), A. Asgarov (Asgarov, 2011, 2016) and other botanists in species spread in Azerbaijan in their works, the biomorphological diversity of the species, its intrinsic systematics have not been studied extensively, and the status of rare species has

not been evaluated in accordance with international requirements.

Herbs annual or perennial. Stem erect or climbing by means of tendrils, winged or wingless. Leaves paripinnate, with rachis terminating in a branched or simple tendril or a bristle, rarely phyllodic or reduced to modified stipules; stipules sagittate or semisagittate, usually smaller than leaflets, sometimes large and leaflike; leaflets 1- to many paired, elliptic, ovate, ovate-oblong, lanceolate, or linear; veins parallel, pinnate, or reticulate. Inflorescence an axillary, pedunculate, 1- to many flowered racemes. Calyx campanulate, unequally or equally toothed; teeth not leaflike, at least 2 teeth less than 2 as long as tube. Corolla purple, pink, yellow, or white, sometimes crimson, brick red, or orange. Staminal tube not oblique at apex; filaments filiform or distally dilated. Style linear or distal dilated, dorsally compressed, pubescent on upper side. Legume laterally compressed, dehiscent. Seeds 2 to many (Cherepanov, 1995; Davis, 1979; Maxted et al., 1988; Shehadeh, 2011; Yakovlev et al., 1996).

Cytological investigations have shown that all species were diploid with $2n = 14$ chromosomes, the basic chromosome number of $x=7$ is constant throughout the genus and that most of the species are diploid, with poliploids as rare exceptions (Leht, 2009; Badr, 2007; Sarker et al., 1997; Yamamoto et al., 1984).

MATERIALS AND METHODS

The materials for the study were collected during expeditions under the leadership of A.M.Asgarov at the Institute of Genetic Resources in 2016. Herbarium data and extensive literature information have also been analyzed. Comparative morphological, botanical - geographical, biomorphological, ecological, taxonomic, floristic- system-

atic, phytocenological methods were used in the study. In addition, the materials stored in the Herbarium Foundation of the Botanical Institute of ANAS and the Herbarium Foundation of the Institute of Genetic Resources of ANAS were investigated. The spreading of species has been given on the regionalization scheme adopted in the flora of Azerbaijan.

A.Asgarova's (Asgarov, 2011, 2016) classification was used for species nomenclature, and Ch. Raunkier (Raunkier, 1937) and I.Serebryakov's (Serebryakov, 1964) classifications were used while analyzing life forms and other ecological features.

RESULTS AND DISCUSSION

In order to explore the *Lathyrus* species spread in the study area, 18 stations located in various areas differentiated from one - another by certain eco-geographical features were chosen and each route was encoded.

Below is a brief description of the types of herbarium and seeds collected.

1. *Lathyrus aphaca* L. An annual plant.

2n = 14.

Distribution: All across Azerbaijan – GC (Greater Caucasus), LC (Lesser Caucasus), Kur-Araz, Talish and Nakhchivan.

Biotope: In crops, fields, gardens, roads and fences, bushings, forests, on the edge of irrigation canals, gravel, sands and so on.

2. *L.hirsutus* L. An annual plant. 2n=14.

Distribution: It is observed in GC, Kur-Araz, Talish, Nakhchivan.

Biotope: It grows in the plants, fields, bushes, and forests.

3. *L.miniatus* Bieb ex Stev. A perennial plant. 2n=14.

Distribution: It is found in GC, LC, Kur-Araz, Talish, Nakhchivan.

Biotope: It grows as weed in forests, bushes, edges of forests, fields, mountain meadows, and planting areas.

4. *L. pratensis* L. A perennial plant. 2n=14.

Distribution: It is spread in GC, LC, Kur – Araz, Talish and Nakhchivan.

Biotope: It grows in forests, bushes, gardens, edges of forests, fields, mountain meadows, and planting areas.

5. *L. laxiflorus* (Desf) O. Kuntze (*Orobis hirsutus* L.) – A perennial plant. 2n=14.

Distribution: It is spread in GC, LC and Talish.

Biotope: It grows in forests and bushes.

6. *L. tuberosus* L. A perennial plant. 2n=14.

Distribution: It is spread in GC, Kur – Araz and Nakhchivan.

Biotope: It grows in crops, vineyards, hedges and fields, irrigation canals, and bushes.

7. *L. sphaericus* Retz. An annual plant. 2n=14.

Distribution: It is spread in GC, LC, Kur – Araz, Talish and Nakhchivan.

Biotope: It grows in forests, bushes, on the edges of forests, stony slopes, and irrigation canals.

8. *L. cicera* L. An annual plant. 2n=14.

Distribution: It is found in GC, LC, Kur – Araz, Talish and Nakhchivan.

Biotope: It grows in gardens, vineyards, planting areas, fields, bushes, around irrigation canals, weed places, and river banks.

9. *L. annuus* L. An annual plant. 2n=14.

Distribution: It is found in GC, Kur – Araz and Talish.

Biotope: It grows in planting areas, gardens, on the edges of forests, bushes, roads and river banks.

The range of species is mapped through the DIVA-Gis software (Figure 1).

Some eco-geographical information of the species collected are given in the table below.

The ecological-botanical analysis of species collected in the study area was conducted on descriptors, ecological information – information on geographical latitude and longitude, height above sea level, type of rock, the degree of slope, and the edge of the slope is given.

In the ecological evaluation of the climatic parameters of *Lathyrus* species, it was determined that *L. hirsutus* was collected in the territory of Imishli region on minimum altitude height (10 m), and in Shahbuz region on maximum altitude, *L.miniatus* and *L. pratensis* (2253 m) from the Batabat plain.

The amount of annual precipitation, temperature (T_{min1} - minimum temperature in January, T_{max7} - maximum temperature for July and T_{oi} – average annual temperature) is based on PAST computer software and is as follows (Figure 2).

It was determined on average annual precipitation that *L. cicera* (345 mm) was collected from the territory of Mistan village, Lerik region on minimum precipitation and *L. miniatus* (612 mm) was collected from Khoshbulah village, Dashkesen region on maximum precipitation.

L.miniatus and *L. pratensis* (-11°C) were observed in Shahbuz region, Batabat pasture on minimum temperature in January and *L.aphaca*, *L. hirsutus* (30.3°C) in Bilasuvar region, Bilasuvar – Iran highway, *L.aphaca*, *L.hirsutus* (30.3°C) on maximum temperature. Minimum average annual temperature was in Shahbuz region, Batabat pasture (-0,2°C), and maximum average annual temperature was in Bilasuvar – Iran highway (9.4°C).

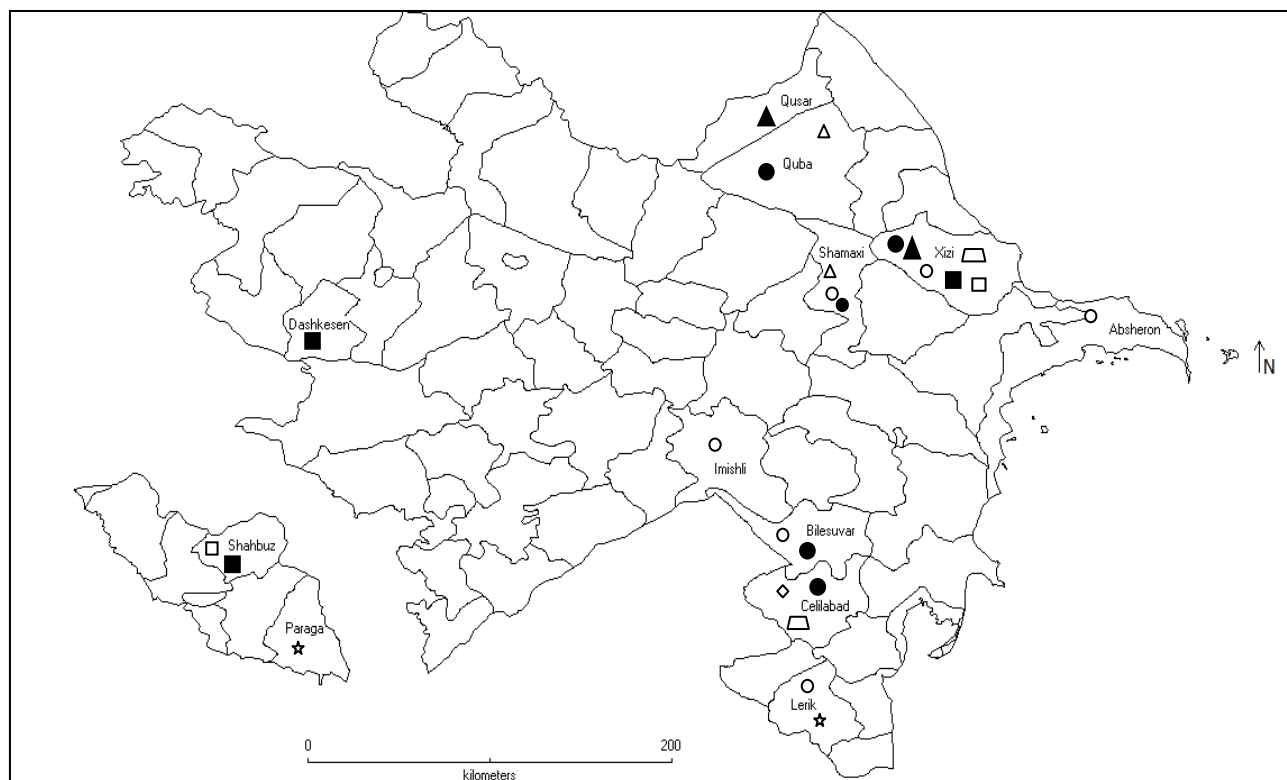


Figure 1. Areas of distribution of species which herbarium and seeds are collected
 ● - *L. aphaca*; ○ - *L. hirsutus*; ■ - *L. miniatus*; □ - *L. pratensis*; ▲ - *L. laxiflorus*;
 △ - *L. tuberosus*; ▭ - *L. sphaericus*; ☆ - *L. cicera*; ◇ - *L. annuus*;

Table 1. Coordinates of *Lathyrus* species found and ecological information on them

Code of place where it was collected	Name of species collected	Date of collection	Place of collection and biotope	Type of rock	Degree of slope	The edge of the slope	Type of soil	Geographic latitude and longitude	The height from sea level (m)
1	2	3	4	5	6	7	8	9	10
AZE16St ₁	<i>Lathyrus miniatus</i>	19.04.16	Khizi region, upper part of Bayahmadyurd village	-	E 6	N	CY	N 40°55'142; E 48°57'280	1079
AZE16St ₃	<i>L. miniatus</i>	19.04.16	Khizi region, between the village of Bakhishli	-	L 2	S	CY, SA	N 40°54'908; E 49°00'928	602
AZE16Ab ₁	<i>L. hirsutus</i>	24.05.16	Absheron district, Institute of Agriculture	-	L 2	-	SC	N 40°31'951; E 049°52'576	12,5
AZE16A ₂	<i>L. hirsutus</i>	09.06.16	territory of Imishli region	BA	L 2	-	CY	N 39°45' 222; E 047° 53' 896	10
AZE16G ₅	<i>L. hirsutus</i> , <i>L. aphaca</i> ,	09.06.16	Bilasuvar region, Bilasuvar – Iran highway	-	L 2	-	CG	N 39° 26' 463; E 48° 31' 126	13
AZE16A ₈	<i>L. aphaca</i> , <i>L. annuus</i>	12.06.16	Jalilabad region, Sulucheshma village.	-	U 3	S	CY	N 39° 12' 406; E 048° 25' 630	139
AZE16B ₁	<i>L. hirsutus</i>	22. 06.16	Lerik region, Galasar village	-	E 6	W	SA	N 38°41'415; E 048°23'790	1350
AZE16B ₂	<i>L. cicera</i>	24. 06.16	Lerik region, Mistan village	BA	U 3	E	GR	N 38°39'003; E 048°24'940	1723
AZE16Q ₄	<i>L. aphaca</i>	14.07.16	Guba region, Digah village	-	R 4	S	SC	N 41°22'324; E 048°30'161	658
AZE16Q ₁	<i>L. aphaca</i>	12.07.16	Guba region. Vladimirovka village	-	O 5	S	SC; GR	N 41° 23' 08; E 048° 32' 211	545

Continued table 1

1	2	3	4	5	6	7	8	9	10
AZE16Q ₅	<i>L. laxiflorus</i>	14.07.16	entrance of Gusar region	-	L 2	-	SC	N 41° 26' 067; E 048° 27' 090	675
AZE16E ₅	<i>L. miniatus</i> , <i>L. sphaericus</i> <i>L. laxiflorus</i> <i>L. aphaca</i>	05.07.16	Khizi region, bottom part of Vardah village, way to Dubrar pasture	-	O 5	N	CY	N 40° 53' 402; E 48° 56' 959	991
AZE16E ₆	<i>L. hirsutus</i>	06.07.16	Khizi region, the edge of way to Altigaj, bank of Atachay	BA	O 5	W	CY	N 40° 53' 07; E 48° 57' 06	927
AZE16E ₈	<i>L. pratensis</i>	06.07.16	Khizi region, territory of Chistiyy – Klyuch	L	E 6	N	CY	N 40° 49' 27; E 48° 52' 43	1529
AZE16D ₃	<i>L. miniatus</i>	13.07.16	Dashkesen region, Khosbulag village	-	O 5	W	SC	N 40° 30' 719; E 046° 05' 012	1527
AZE16D ₆	<i>L. tuberosus</i> , <i>L. hirsutus</i> <i>L. aphaca</i>	17.07.16 03.07.16	Shamakhi region, Shamakhi – Aghsu road	-	L 2	W	SC	N 40° 38' 550; E 048° 28' 450	794
AZE16E ₁	<i>L. cicera</i>	21.07.16	Ordubad region, Paragha village	L	E 6	S	CY	N 39° 5' 10; E 45° 55' 13	1644
AZE16E ₂	<i>L. pratensis</i> , <i>L. miniatus</i>	22.07.16	Shahbuz region, Batabat pasture, surrounding of Lake Batabat	L	O 5	S	SC	N 39° 32' 4; E 45° 47' 23	2253

Type of rock: BA - basalt; L- granite;

Degree of slope: L 2 - flat 0°-3°; R 4 – hill 8-16%; U 3 – wavy 3°-8°; O 5- mountainous 3°-45°; E 6- steep slope >45°;

The edge of the slope: S – south; W – west; N – north; E – east;

Type of soil: clay – CY; clay-gravel – CG; sandy – SA; sandy-clay – SC; gravel – GR.

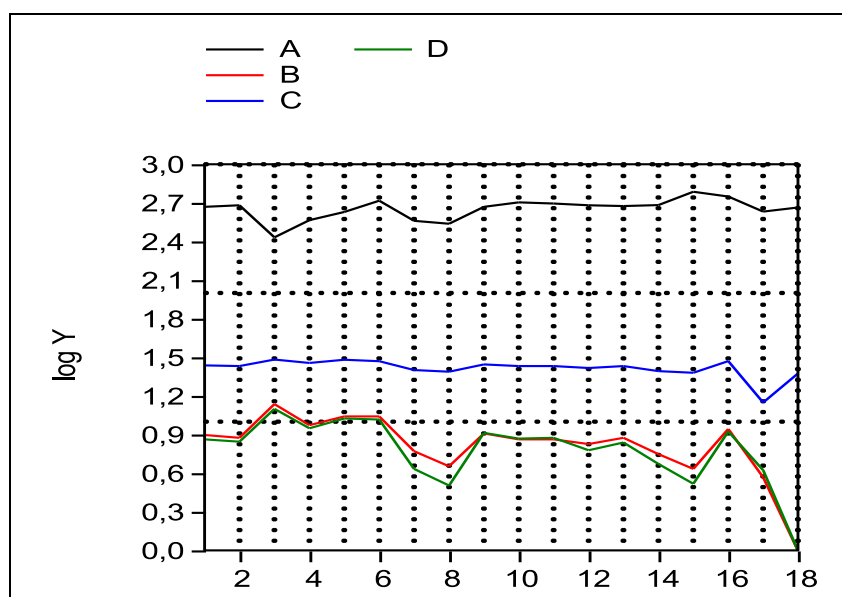


Figure 2. Relation of collected species to precipitation and temperature

A – amount of precipitation; B - T_{\min} ; C - T_{\max} ; D- T_{or}

Different herbarium samples collected by use for the fenetic (taximetric) analysis of *Lathyrus* species were reviewed. At least two examples of each population have been studied and each population is labeled as the single Operational Taxonomic Unit (OTU). For the morphological analysis of plants, 8 quantitative characteristics (plant height, number of leaflets, length, width, number of legumes, length, width, number of seeds) were selected.

The measurements were made at least 2-3 copies of each population, and the average value was calculated. Based on the results obtained, the taximetric (fenetic) analysis was carried out using the method of Cluster Analysis (CA). The analyzes were conducted through the SSPS Win (SPSS version 16.0) software.

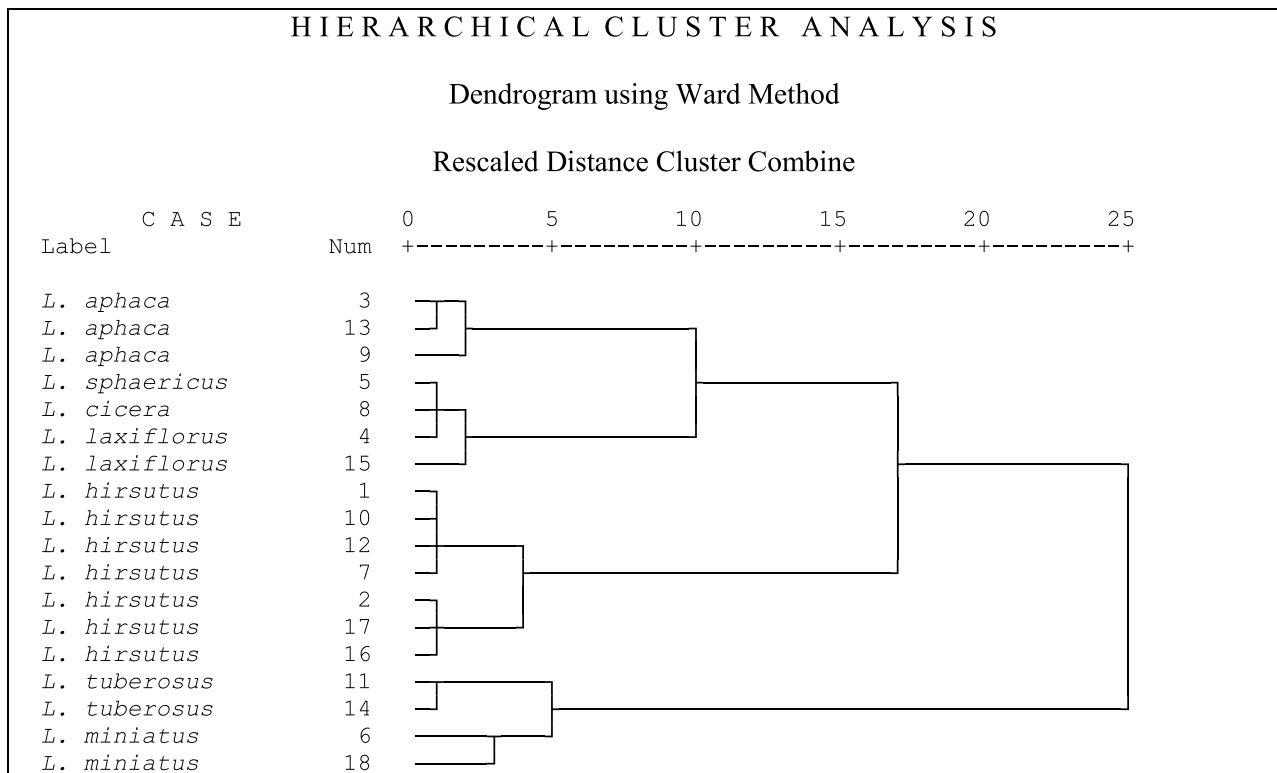


Fig. 3. Single Integrated Cluster Analysis of morphological signs in *Lathyrus* species.

The taximetric (fenetic) relationship between the *Lathyrus* species is shown in the Fig 3. Three main clusters were observed at the 15th level. The first cluster is composed of *L. aphaca*, *L. sphaericus*, the second cluster *L. cicera*, *L. laxiflorus*, and *L. hirsutus*. The third cluster is associated with *L. tuberosus*, *L. miniatus*, spread across different areas. Three main clusters were differentiated from one – another for their signs.

The first cluster is divided into 2 subgroups. The first and second subgroups are characterized by *L. aphaca* samples.

The second cluster is divided into 4 subgroups. The first subgroup includes *L. sphaericus*, *L. laxiflorus* and *L. cicera*. There are different opinions about the status of this species. According to Chefranov (Chefranov, 1971, 1987) *L. cicera* - *Cicercula* (Medik) includes in Czeffr.sub – genus and the other species belong to *Orobis* (L.) Peterm. sub-genus. According to the system of Kupicha (Kupicha, 1974, 1983), Asmussen and Liston (Asmussen and Liston, 1998) it is included in the sections of *L. sphaericus* - *Lineacarpus*, *L. cicera* - *Lathyrus*, *L. laxiflorus* – *Pratensis*. The species are concentrated in this subgroup due to the similarities in the morphological signs (the height of the plant and the number of leaflets). Further research is required to be conducted in order to confirm this area. The second subgroup includes *L. laxiflorus*. The third and fourth subgroups relates to *L. hirsutus* samples spread across different territories.

The third cluster is divided into 2 subgroups. The first subgroup includes *L. tuberosus*, second subgroup *L. miniatus* samples. These species belong to the *Lathyrus* subgenus. The species is concentrated in the *Lathyrus* section, depending on the system of Kupicha (Kupicha, 1974, 1983), Asmussen et Liston (Asmussen et Liston, 1998). According to Chefranov (Chefranov, 1971, 1987), the species belong to the *Lathyrus* subgenus.

In our research, species are in line with the systematic proposed by Chefranov (Chefranov, 1971, 1987).

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Azərbaycanda Gülülçə (*Lathyrus* L. s. l.) Növlərinin Yayılması Və Biomorfoloji Müxtəlifliyinin Qiymətləndirilməsi

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2016-cı ildə təbiətdə aparılan monitorinqlər nəticəsində Azərbaycanda 18 marşrut üzrə Gülülçə (*Lathyrus* L. s. l.) cinsinin 9 növü, 13 populyasiyanı əhatə edən toxum və 80 herbari nüsxəsi toplanılmışdır. Məqalədə onların descriptor məlumatları əsasında biomorfoloji müxtəlifliyinin qiymətləndirilməsi və yayılması haqda məlumat verilir.

Açar sözlər: Gülülçə, növ, cins, Azərbaycan, ekoloji-botaniki

**Распространение Видов Рода *Lathyrus* L. s.l. В Азербайджане И Оценка
Их Биоморфологического Разнообразия**

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В результате мониторингов, проведенных в природных условиях в 2016 году в Азербайджане, были собраны 13 образцов семян и 80 гербарных экземпляров, относящихся к 9 видам рода Чины (*Lathyrus* L.s. l.). В статье приводятся результаты исследований по оценкам биоморфологического разнообразия и распространению видов чины на основе дескрипторных данных.

Ключевые слова: *Lathyrus* L., вид, род, Азербайджан, эко-ботанический

Evaluation of Biomorphological Diversity and Distribution of Vetch (*Vicia* L.) Species in Azerbaijan

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The article presents the results of the study of the estimation of the biomorphological diversity of 12 *Vicia* L. species covering 33 populations. Expedition materials and descriptor information collected by authors in various botanical and geographical regions of Azerbaijan are analyzed.

Keywords: *Vetch*, biomorphological diversity, species, genus, areal

INTRODUCTION

Vetch (*Vicia* L.) is one of the most widely spread genera of the *Fabaceae* Lindl. of the *Magnoliopsida* class. The species included into the genus spread across the northern hemisphere, including the middle mountain ranges in Azerbaijan. *Vicia* was first described by K. Linney (Linneaus, 1753). There are 200 species of vetch (*Vicia* L.) (Tsvelyov, 1987) in the world flora, and 41-43 species of vetch in Azerbaijan (Asgarov, 2011). B.A. Fedchenko grouped 83 species of vetch for the USSR flora into 3 subgenera, 4 sections, 31 rows; 41 species of vetch of Azerbaijan were grouped into 3 subgenera, 20 rows (Fedchenko, 1948). In the "Caucasian Flora" it has been noted that there are 150 species of vetch in the world, 48 wild species and one cultivar in Caucasus (Grossheim, 1952). Caucasian species were studied by A.Radghi (Radghi, 1971) and N.Tsvelyov worked with the vetch species of the eastern part of Europe (Tsvelyov, 1987). Turkish species were studied by P.Davis and U.Plitman (Davis, Plitmann, 1970). The only taxonomic study on the whole genus was conducted by F. K. Kupicha at the world level (Kupicha, 1973, 1975, 1976). Although in the multivolume "Flora of Azerbaijan" 41 wild species and 1 cultivar have been described, in "The plant world of Azerbaijan" of A. Asgarov the information on 43 wild species and 1 cultivar have been given (Asgarov, 2016). The varietal systematic biomorphological diversity of genus has not been widely studied and evaluation of the status of rare species on international standards has not been carried out.

MATERIALS AND METHODS

In 2017, in the Department of Ecobotanics and Systematics of the Institute of Genetic Resources, under the guidance of A. Asgarov at the expeditions 12 species, 33 populations of herbarium and seeds

were collected and studied. Moreover, collections of the Herbarium Funds of the Institute of Botany of ANAS, the Genetic Resources Institute (AGRI) and the Institute of Botany of the Republic of Georgia (TBI) were studied as a research material. The literature and Internet data have been analyzed. The definition of the nomenclature issues is based on The International Botanical Code (Austria, Vienna, 2005; Allkin et al., 1986) In the determination of species and identification of their nomenclature "Flora of Azerbaijan" and A. Asgarov's books were used (Asgarov, 2011, 2016), in the analysis of other features Ch. Raunkier (Raunkiaer, 1937) and I.Serebryakov's classification (Serebryakov, 1964) were used. Comparative morphological (Gunn and Kluge, 1969; 1970; 1976) floristic (Tupikova, 1926), biomorphological, sistematic (Maxted, 1990, 1995) phytocenological and experimental methods (Leht, 2005) were used in the research.

RESULTS AND DISCUSSION

Vetch (*Vicia* L.) is one of the highest quality, two and perennial fodder grass. Morphological signs are important in systematic of vetch species, as well as in the design of the prescribed keys. The main characteristic of the genus is the column, correlation of crown and calyx lobe, and the leaf finish with clasper. The calyx tube is usually curved from the base. The vexillum is hollow or full. The wing petals are joined to slipcover. The column is thoroughly hirsute or unilateral bearded on the top, rarely naked.

Species are distinguished by the color of the crown, the shape of the flower, the characteristics of the leaf and stipule, the fruit and seeds.

The leaves are pair featherlike, ends with many or less branched beads, or with a sharp end, sometimes with a single leaf. **Stipules** are often semi-gothic shaped. **The flowers** are 1 or 2-3, lo-

cate in the axillary bud, almost as a sessile or cluster is multi-flowered. The peduncle is longer than the leaves, or equal to it, and sometimes is shorter. **The corolla** is yellow, red, purple, blue, blue, matte or dark purple, red-purple, blue, yellowish-orange, bright purple, pale blue, bright blue and so on in colors. **Calyx** is 5 toothed, usually it has 3 upper are longer than 2 down teeth. **The corolla resembles** is a common butterfly structure. 9 adjacent stamens form a pipe, and 1stamen is free. **The vexillum** is undescribed claw, the wings are almost equal to the claw, slipcover are blunt, shorter than the vexillum, sometimes with the same length. **The stem** is gentle or sloppy, externally tight or sparse, sometimes almost naked, flat shield or creeping. **Legume** is on short or long stalk; Legume is too or less squeezed; it is hirsute or sliced; cylindrical, bead shaped. Usually it is multi-seeded, and sometimes it is two-seeded. Legume is soft, fluffy, or

bare. Cytological studies show that the genus has a chromosome set of $2n=10, 12, 14, 16, 18, 28$.

The distribution of the vetch species is based on five major botanical-geographical regions of Azerbaijan: 1. Greater Caucasus, 2. Lesser Caucasus, 3. Kura-Araks, 4. Talysh region, 5. Nakhchivan. The results of our research show that the vetch is more widely spread in the Greater Caucasus region of Azerbaijan (34 species). From the species of vetch 27 spread in Talysh region, 26 in the Lesser Caucasus, 24 in Nakhchivan, and 12 species in Kura-Araks. You can see this in the table blow (Table 1).

33 routes were selected in different regions of Azerbaijan for exploration of vetch species, distinguished by certain bioecological characteristics, and were coded for identification. Range maps of the collected species were compiled using DIVA-GIS computer program (Figure 1).

Table 1. Distribution of *Vicia* L. species in botanical-geographical regions

No	Name of species	Botanical-geographical regions				
		Greater Caucasus	Lesser Caucasus	Kura-Araks	Talysh	Nakhchivan
1	2	3	4	5	6	7
1	<i>Vicia abbreviata</i> Fisch.ex Spreng. (<i>V.truncatula</i> Fisch.ex Bieb.)	+	+	+	+	+
2	<i>V. alpestris</i> Stev.	+				
3	<i>V.amphicarpa</i> Lam.	+	+		+	
4	<i>V. anatolica</i> Turrill (<i>V.hajastana</i> Grossh.)					+
5	<i>V. angustifolia</i> Reichard	+	+	+	+	+
6	<i>V. antiqua</i> Grossh.	+	+			
7	<i>V. balansae</i> Boiss.	+	+			+
8	<i>V. bithynica</i> (L.) L.	+			+	
9	<i>V. boissieri</i> Freyn	+	+		+	
10	<i>V. cappadocica</i> Boiss.et Bal. (<i>V. paucijuga</i> (Trautv.) B.Fedtsch.)				+	+
11	<i>V.cassubica</i> L.	+			+	
12	<i>V.caucasica</i> Ekvtim.	+				
13	○ <i>V. ciceroides</i> Boiss.(<i>V. rafigae</i> Tamamsch.)					+
14	<i>V.ciliatula</i> Lipsky	+			+	
15	<i>V. cinerea</i> Bieb.	+	+	+	+	+
16	<i>V. cordata</i> Wulf. ex Hoppe	+		+	+	+
17	<i>V. crocea</i> (Desf.) Fritsch	+	+		+	
18	<i>V. elegans</i> Guss.		+			+
19	<i>V. ervilia</i> (L.) Willd.	+	+		+	+
20	<i>V. grandiflora</i> Scop.	+	+	+	+	+
21	<i>V. grossheimii</i> Ekvtim.	+	+			+
22	○ <i>V. hololasia</i> Woronow	+	+			
23	<i>V.hirsuta</i> (L.) S.F.Gray	+	+		+	+
24	<i>V. hybrida</i> L.	+	+	+	+	+
25	<i>V. hyrcanica</i> Fisch. et C.A.Mey.				+	+
26	<i>V. iberica</i> Grossh.	+				
27	<i>V. larissae</i> Prima	+				
28	<i>V. lathyroides</i> L.		+		+	
29	○ <i>V. loiseleurii</i> (Bieb.) Litv. (<i>V. meyeri</i> Boiss.)	+			+	
30	<i>V. lutea</i> L.	+			+	+
31	<i>V. narbonensis</i> L. (<i>V. johannis</i> Tamamsch.)	+	+	+	+	+
32	<i>V. nissoliana</i> L.(<i>V.variegata</i> Willd.)		+			+
33	<i>V. pannonica</i> Crantz	+	+	+	+	+
34	<i>V. peregrina</i> L.	+	+	+	+	+
35	<i>V. pilosa</i> Bieb.		+			

Continued table 1

1	2	3	4	5	6	7
36	<i>V. sativa</i> L.	+	+	+	+	+
37	<i>V. semiglabra</i> Rupr.ex Boiss	+				
38	<i>V. serratifolia</i> Jacq.				+	
39	<i>V. sepium</i> L.	+	+			
40	<i>V. tetrasperma</i> (L.) Schreb.	+	+		+	+
41	<i>V. variabilis</i> Freyn et Sint.	+	+	+	+	+
42	<i>V. varia</i> Host (<i>V. dasicarpa</i> auct.)	+	+	+	+	+
43	<i>V. villosa</i> Roth	+				
Total		34	26	12	27	24

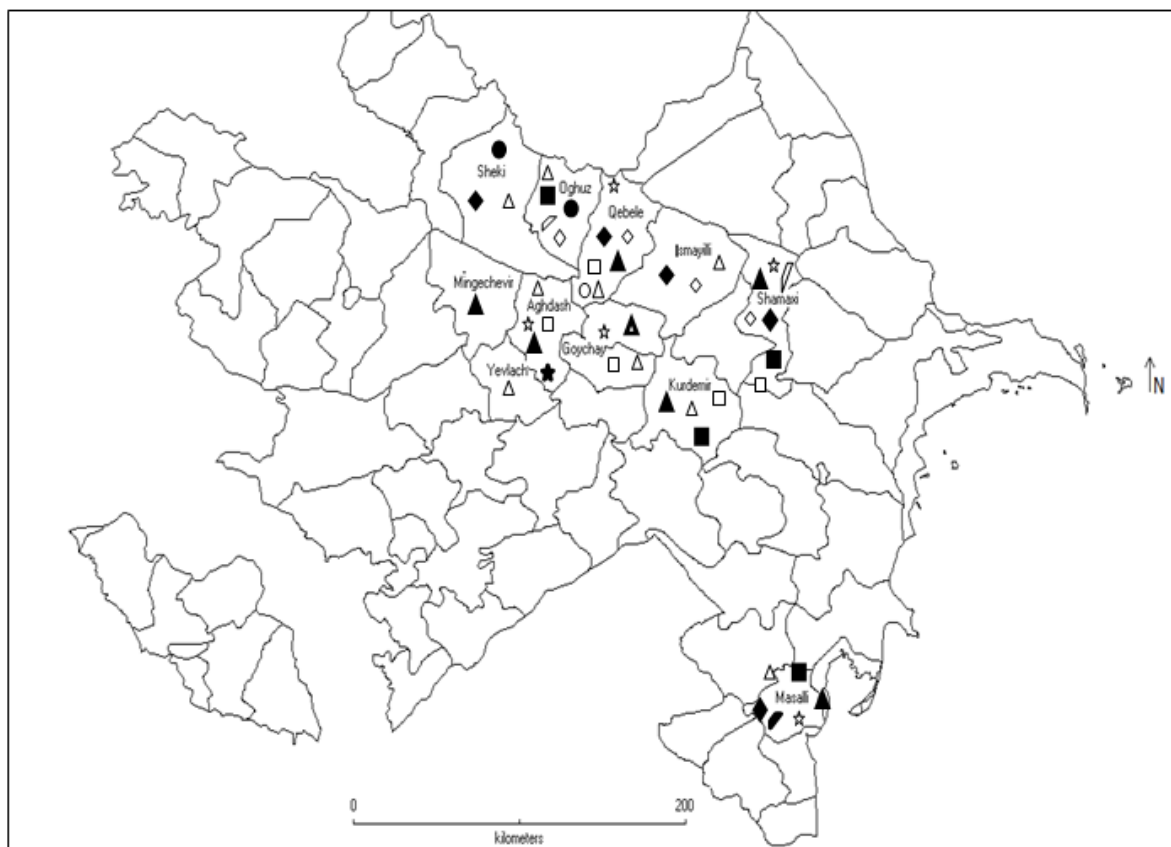


Figure 1. Distribution of *Vicia* L. species in Azerbaijan: Δ - *V. sativa* subsp. *nigra*; \blacktriangle - *V. sativa* subsp. *sativa*; \square - *V. peregrina*; \blacksquare - *V. tetrasperma*; \star - *V. lutea*; \star - *V. monantha* subsp. *monantha*; \blacklozenge - *V. narbonensis*; \diamond - *V. pannonica*; \circ - *V. villosa*; \bullet - *V. abbreviata*; \diagup - *V. tenuifolia* subsp. *variabilis*; \diagdown - *Vicia bithynica*.

As you can see from the table (Table 2) the most types were collected from meadows (17 species), at least types from forest area (4 species).

Ecological assessment of vetch species on climate parameters has been carried out. It was found that in the *min* height were collected *V. lutea*, *V. bithynica* (-25 m) in Masally region, Tekle village territory, *Max* height from Shamakhi city, Pirgulu village, were collected *V. narbonensis*, *V. peregrina*, *V. variabilis* and *V. sativa subsp. nigra* (1430 m).

Due to environmental factors in the biomorphological structure of species have been observed significant changes. Information about the amount of annual rainfall, temperature (T_{\min} minimum temperature, T_{\max} - maximum temperature for month and T_{oi} - average annual temperature) is established by using program DIVA-GIS and is as follow (Scheme1).

The average annual rainfall has been determined by the fact that on *min* rainfall were collected *V. peregrina*, *V. sativa subsp. nigra*, *V. tetrasperma* from territory of Kurdamir region, Karrar village (360 mm), on the maximum rainfall were collected *V. lutea*, *V. narbonensis* from territory of Masally region, Kalinovka village (701 mm). *Min* temperature for January was encountered in territory of Oguz region, Dashagil village (-17.4°C), max temperature interterritory of Geokchay city, Mirzaguseynly village (33.2°C). *Min* average annual temperature was observed in territory of Oguz region, Dashagil village (-7.5°C), max average annual temperature in territory of Yevlach region, Haciselly village (20.5°C).

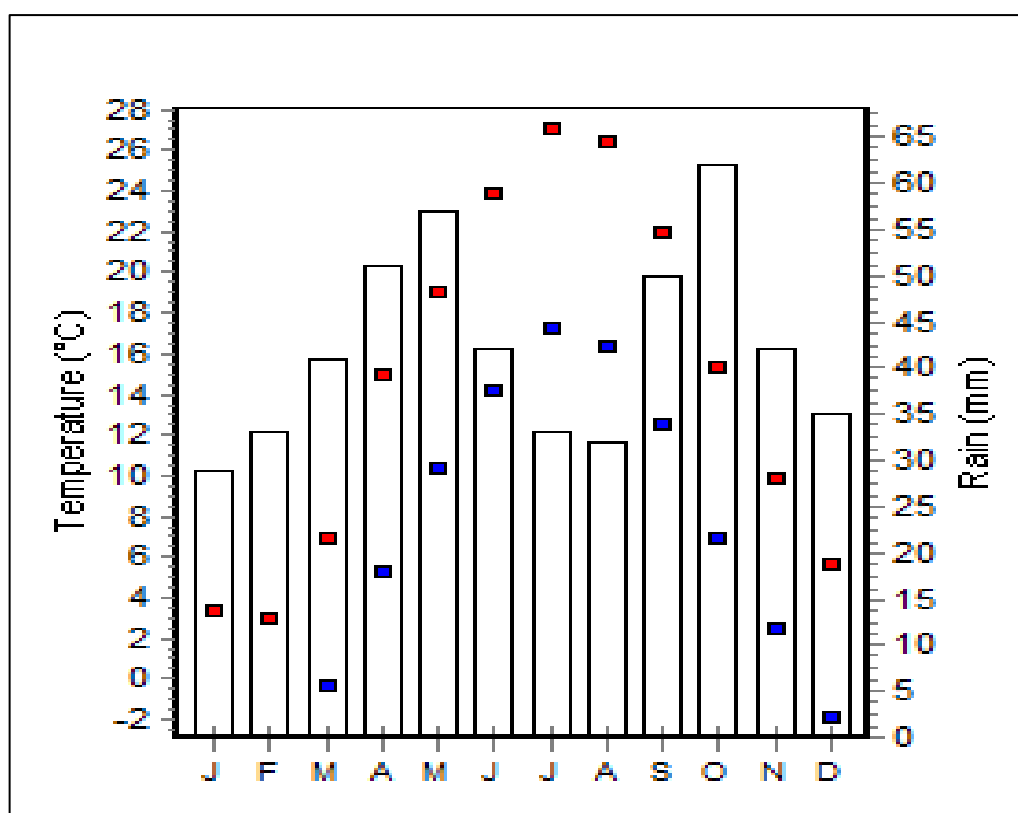
Table 2. Vetch species seeds and herbariums collected from research sites and their ecological-geographical data

Locality code	Name of collected species	Date	Locality and site description	Relief	Slope	ST	Latitude and longitude	Alt (m)
1	2	3	4	5	6	7	8	9
AZE17K2 M1	<i>Vicia sativa</i> subsp. <i>nigra</i> <i>V. sativa</i> subsp. <i>sativa</i>	15.05.2017	Kurdamir region, Garis-Ayriband village, GR	L2	N	SC	N 40°20' 574 E 048° 22' 548	8
AZE17K3 M2	<i>V. peregrina</i> ; <i>V. sativa</i> subsp. <i>nigra</i> <i>V. tetrasperma</i>	15.05.2017	Kurdamir region, Karrar village, WS, around of the swamp	L2	N	SC	N 40° 18' 220 E 048° 16' 162	5
AZE17K4 M3	<i>V. sativa</i> subsp. <i>nigra</i>	16.05.2017	Yevlach region, Haciselly village, GR	L2	S	SA	N 40° 43' 105 E 047° 07' 143	20
AZE17K5 M4	<i>V. sativa</i> subsp. <i>nigra</i>	16.05.2017	Yevlach region, road of Xaldan, RS	L2	S	CY	N 40° 43' 582 E 047° 11' 810	21
AZE17K6 M5	<i>V. sativa</i> subsp. <i>nigra</i>	16.05.2017	Agdash region, Upper Nematabad village, GR	L2	S	SC	N 40° 41' 794 E 047° 18' 360	12
AZE17K7 M6	<i>V. sativa</i> subsp. <i>sativa</i>	16.05.2017	Mingachevir city, the 7th km of road Chaldan, GR	L2	W	CY	N 40° 44' 260 E 047° 08' 460	28
AZE17K9 M7	<i>V. lutea</i> , <i>V. monantha</i> subsp. <i>monantha</i> , <i>V. peregrina</i> , <i>V. sativa</i> subsp. <i>sativa</i> , <i>V. tetrasperma</i>	17.05.2017	Agdash region, Agchayazi village, the right bank of Turyanchay	O5	S	SC	N 40° 42' 972E 047° 33' 010	107
AZE17K10 M8	<i>V. sativa</i> subsp. <i>sativa</i>	17.05.2017	Agdash region, Xosrov village, GR	L2	E	SC	N 40° 38' 277 E 047° 34' 847	34
AZE17K11 M9	<i>V. lutea</i> <i>V. peregrina</i>	17.05.2017	Geokchay city, Mirzaguseynly village, Upper-Shirvan kanal, RS	L2	N	SC	N 40° 38' 871 E 047° 37' 512	55
AZE17K12 M10	<i>V. sativa</i> subsp. <i>nigra</i> <i>V. sativa</i> subsp. <i>sativa</i>	18.05.2017	Geokchay city, Bygyr village, GR	O5	W	SC	N 40° 37' 773 E 047° 53' 674	151
AZE17K13 M11	<i>V. lutea</i> <i>V. sativa</i> subsp. <i>nigra</i>	18.05.2017	Geokchay city, Karayazy village, GR	O5	N	CY	N 40° 37' 186 E 047° 59' 107	173
AZE17K14 M12	<i>V. narbonensis</i> <i>V. pannonica</i> <i>V. sativa</i> subsp. <i>nigra</i> <i>V. sativa</i> subsp. <i>sativa</i>	18.05.2017	Ismaily region, Kurdmashy village, GR	O5	N	CY	N 40° 38' 322 E 048° 03' 195	268
AZE17K15 M13	<i>V. lutea</i> <i>V. sativa</i> subsp. <i>nigra</i>	18.05.2017	Geokchay city, Karamaryam village, the grain area of Chermodil, GR	O5	W	CY	N 40° 35' 303 E 048° 01' 146	199
AZE17K16 M14	<i>V. sativa</i> subsp. <i>nigra</i>	19.05.2017	The right side of road Shamakhy - Akhsu	O5	W	SA	N 40° 36' 233 E 048° 26' 209	739
AZE17K17 M15	<i>V. lutea</i> <i>V. pannonica</i> <i>V. sativa</i> subsp. <i>sativa</i>	19.05.2017	Shamakhy city, Sagyan village, GR	E6	N	CY	N 40° 38' 912 E 048° 29' 266	745
AZE17K18 M16	<i>V. lutea</i> , <i>V. pannonica</i> , <i>V. narbonensis</i> , <i>V. peregrina</i> <i>V. sativa</i> subsp. <i>nigra</i> , <i>V. tenuifolia</i> subsp. <i>variabilis</i> , <i>V. sativa</i> subsp. <i>sativa</i>	19.05.2017	Shamakhy city, Madrasy village, GR	O5	N	SC	N 40° 38' 650 E 048° 36' 061	696
AZE17K19 M17	<i>V. narbonensis</i> , <i>V. peregrina</i> , <i>V. variabilis</i> , <i>V. sativa</i> subsp. <i>nigra</i>	20.05.2017	Shamakhy city, Pirgulu village, FO	E6	S	SC	N 40° 46' 864 E 048° 36' 168	1430
AZE17K20 M18	<i>Vicia peregrina</i> , <i>V. pannonica</i> , <i>V. narbonensis</i> , <i>V. lutea</i> , <i>V. sativa</i> subsp. <i>nigra</i> , <i>V. sativa</i> subsp. <i>sativa</i> , <i>V. villosa</i> subsp. <i>varia</i>	20.05.2017	Shamakhy city, Mirzandiya village, GR	L2	S	SC	N 40° 34' 737 E 048° 43' 648	584
AZE17Z1 M19	<i>V. pannonica</i> , <i>V. sativa</i> subsp. <i>sativa</i>	19.06.2017	Kabala region, Lesser Amily village, GR	O5	E	SC	N 40° 84' 509 E 047° 79' 514	381
AZE17Z3 M20	<i>V. pannonica</i> , <i>V. villosa</i> subsp. <i>varia</i>	19.06.2017	Kabala region, Lesser Piraly village, GR	O5	N	SC	N 40° 92' 637 E 047° 76' 994	552
AZE17Z4 M21	<i>Vicia narbonensis</i> , <i>V. pannonica</i> , <i>V. sativa</i> subsp. <i>nigra</i>	20.06.2017	Kabala region, Yenikand village, GR	E6	S	SC	N 40° 84' 938 E 047° 85' 043	589
AZE17Z6 M22	<i>V. pannonica</i>	21.06.2017	Oguz region, Bayan village, RS	L2	W	S C	N 41° 03' 521 E 047° 44' 025	463

Continued Table 2

1	2	3	4	5	6	7	8	9
AZE17Z7 M23	<i>V. abbreviata</i> , <i>V. variabilis</i> , <i>V. sativa</i> subsp. <i>nigra</i>	21.06.2017	Oguz region, Dashagil village, FO	O5	N	SC	N 41° 14' 513 E 047° 42' 252	1010
AZE17Z9 M24	<i>V. lutea</i> , <i>V. narbonensis</i> , <i>V. pannonica</i> , <i>V. peregrina</i> , <i>V. sativa</i> subsp. <i>nigra</i>	22.06.2017	Kabala region, Amirvan village, WS	O5	S	CY	N 40° 81' 906 E 047° 88' 421	535
AZE17Z10 M25	<i>V. sativa</i> subsp. <i>nigra</i> , <i>V. tetrasperma</i>	23.06.2017	Oguz region, Muchas village, WS	O5	N	GR	N 41° 09' 619 E 047° 36' 447	708
AZE17Z11 M26	<i>V. abbreviata</i>	23.06.2017	Sheki region, Kysh village, FO	E6	S	SC	N 41° 25' 885 E 047° 18' 615	995
AZE17Z12 M27	<i>V. narbonensis</i> , <i>V. sativa</i> subsp. <i>nigra</i>	23.06.2017	Sheki region, Juma village, FO	O5	W	GR	N 41° 22' 496 E 046° 89' 632	262
AZE17Ma1 M28	<i>V. sativa</i> subsp. <i>sativa</i>	12.07.2017	Masally region, Sharafa village, RS	L2	N	CY	N 39° 05.205 ' E 48° 67.377 '	-15 d.s.h
AZE17Ma2 M29	<i>V. sativa</i> subsp. <i>nigra</i> <i>V. tetrasperma</i>	12.07.2017	Masally region, Shychlar village, around of the Shychlar river	O5	W	SC	N 38° 58.48 ' E 48° 33.54 '	98 d.s.h
AZE17Ma3 M30	<i>V. sativa</i> subsp. <i>nigra</i>	13.07.2017	The road of Masally – Yardymly, RS	O5	S	SC	N 38° 57.43 ' E 48° 31.19 '	154 d.s.h
AZE17Ma5 M31	<i>V. bithynica</i> <i>V. lutea</i>	13.07.2017	Masally region, Tekle village, around of the railway station, RS	L2	S	SY	N 39° 07.41 ' E 48° 40.08 '	-25 d.s.h
AZE17Ma6 M32	<i>V. bithynica</i> <i>V. lutea</i>	14.07.2017	Masally region, Kyzylagach village, around of the garden area, GR	L2	W	SC	N 39° 03.0 ' E 48° 49.4 '	-23 d.s.h
AZE17Ma7 M33	<i>V. lutea</i> <i>V. narbonensis</i>	14.07.2017	Masally region, Kalinovka village, GR	L2	N	SC	N 39° 2.29 ' E 48° 46.44 '	24 d.s.h

Locality code the code given to the location of collected samples. **Slope** E- east, N- north, S- south, W- west. **Site description** FO – forest, RS- side of road, GR- meadow, WS- bank of river. **Relief** E6- steep slope 30°; L2-plain 0°–3°; O5- foothills 16°–30°, **ST soil texture** CY- alumina; CG-clay-gravel, SA- sandy SC- sandy-clay; GR- gravel



Scheme 1. Average annual rainfall and temperature of collected samples

- - The red square is the maximum temperature indicators (T_{\max})
- - The blue square is the minimum temperature indicators (T_{\min})
- - Monthly precipitation (mm).

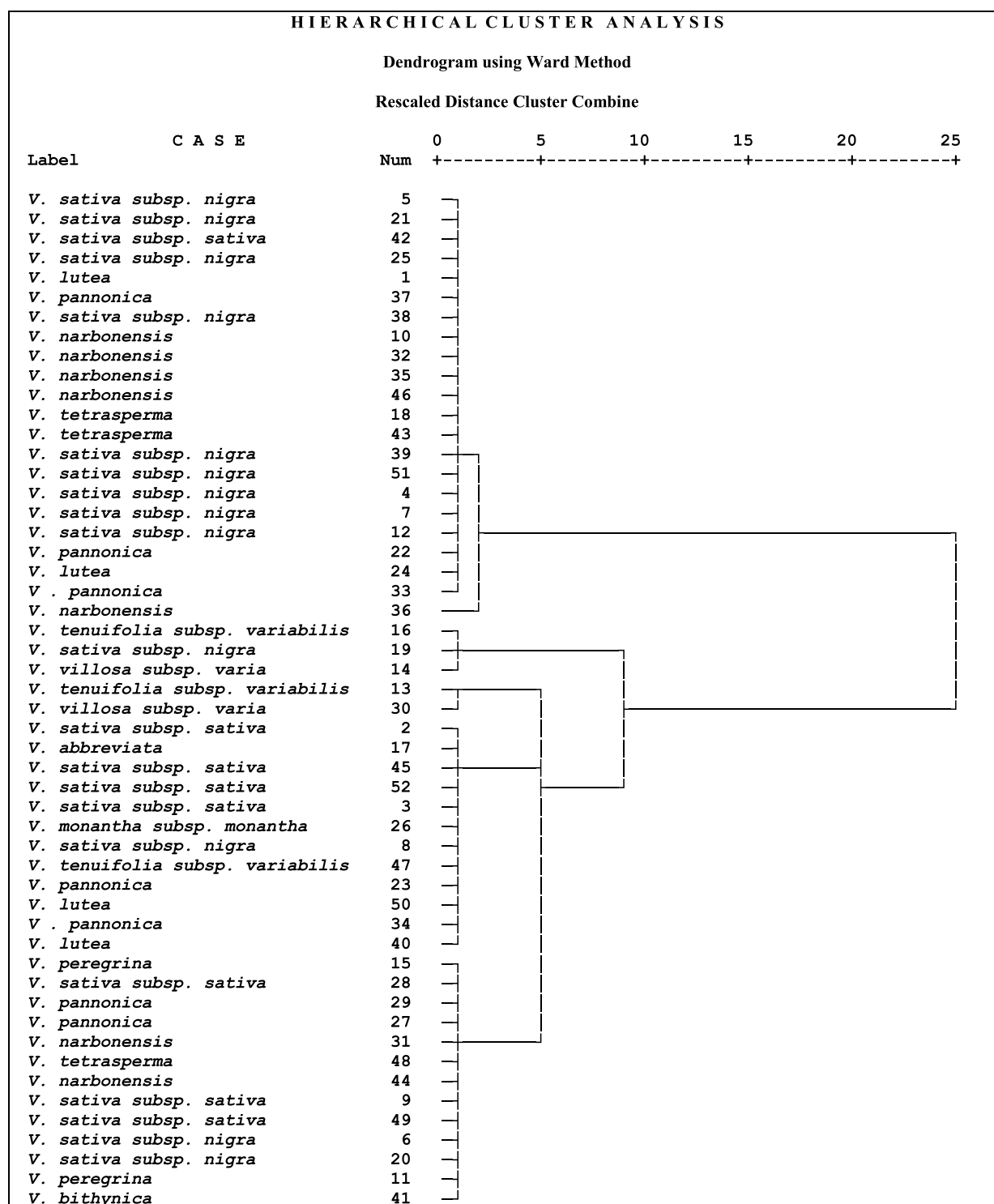


Figure 2. Unified Cluster analysis of morphological characteristics in *Vicia* L. species.

In 2017, 11 species and 33 populations were selected for phenetical (taximetric) analysis of vetch species. At least two samples were studied from each population and each population was marked as Operational Taxonomic Unit (OTU). For the biomorphological analysis, 8 quantitative characteristics (plant height, number, length, width of leaflet, number, length, width of beans, number of seeds) were selected. At least 2-3 sample parameters taken from

each population were measured and the average score was calculated. Based on the results, by using the Cluster Analysis method was carried out a taximetric analysis. The analyses were conducted through the SPSS Win (SPSS 16.0) program. The phenomenon (taximetric) relationship between *Vicia* L. species is shown in the following figure (Figure2). As we can see from the table 3 main clusters observed at the 12 level.

The first main cluster is divided to 22 groups: *V. sativa* subsp. *nigra* (4,5,7,12,21,25,38,39,51), *V. lutea* (1,33), *V. sativa* subsp. *sativa* (42), *V. panonica* (22, 33, 37), *V. narbonensis* (10,32,35,36, 46), *V. tetrasperma* (18,43) which belongs to subgenus *Vicia* (according to Tsvelyov).

The second cluster is related to *V. tenuifolia* subsp. *variabilis* (16), *V. sativa* subsp. *nigra* (19), *V. villosa* subsp. *varia* (14) which belongs to section *Cracca* (according to Fedchenko).

The third main cluster is composed of *V. tenuifolia* subsp. *variabilis* (8, 13), *V. villosa* subsp. *varia* (2, 30, 47), *V. abbreviata* (17), *V. sativa* subsp. *sativa* (3, 9, 28, 45, 49, 52), *V. monantha* subsp. *monantha* (26), *V. panonica* (23, 27, 29, 34), *V. lutea* (40, 50), *V. peregrina* (11, 15), *V. narbonensis* (31, 44), *V. sativa* subsp. *nigra* (6, 20), *V. bi-thynica* (41) which belongs to section *Hypechusa*.

In our research three main clusters are differentiated from each other for having characters: the height of the plant, the number of leaflets, the number of legumes and the number of seeds.

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**Azərbaycanın Lərgə (*Vicia* L.) Növlərinin Yayılması və Biomorfoloji
Müxtəlifliyinin Qiymətləndirilməsi**

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Məqalədə 2017-ci ildə Azərbaycanın müxtəlif bölgələrindən toplanılmış 12 növə, 33 populyasiyaya aid lərgə növləri, onların deskriptor məlumatları əsasında yayılması və biomorfoloji qiymətləndirilməsi nəticələri verilmişdir.

Açar sözlər: *Lərgə, biomorfoloji müxtəliflik, növ, cins, areal*

**Распространения Видов Рода *Vicia* L. в Азербайджане и Оценка
Их Биоморфологического Разнообразия**

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В статье приводятся результаты исследования оценки биоморфологического разнообразия видов рода *Vicia* L. (12 видов, 33 популяции). Анализируются экспедиционные материалы и данные дескрипторов, собранные авторами в различных ботанико-географических районах Азербайджана.

Ключевые слова: *Вика, биоморфологическое разнообразие, вид, род, ареалы*

Dynamics Of Changes Of Inflammation Markers Depending On The Duration Of Toxicosis

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The aim of the research was to study the changes in the dynamics of inflammation during exotoxicosis. The study was conducted on 16 chinchilla rabbits. The model of exotoxicosis was made in a special chamber by inhalation with HCl vapors. The blood taken from the animals of the experimental group was examined to determine the content of peptide molecules, total bilirubin, creatinine and the amount of thymol. It has been revealed that exogenous intoxication primarily causes inflammation in the body, and then this process turns into endogenous intoxication.

Keywords: Toxicity, exogenous intoxication, total bilirubin, creatinine

INTRODUCTION

Currently, the accelerated rate of environmental changes has a toxic effect on the living organism. And this creates an inadequate environment for the normal functioning of cells. In the atmosphere, the concentration of certain substances, including heavy metals (Berezin, 2014; Guskova et al., 2014; Kazimov, 2014), has been increased. Some chemical substances, as well as chemical compounds that enter the body through the respiratory tract, create a toxic state. The liver reacts first to this condition, so the number of hepatitis increases (Agzamova and Aliyeva, 2009; Baymatov et al., 2014; Kiselyova et al., 2009). Despite the numerous scientific works in this field, the pathogenesis of functional and structural changes in the liver under exogenous intoxication has been studied by inaccuracy (Bryukhin et al., 2004; Khrebetovskiy, 2007; Baymatov et al., 2014).

The purpose of our study was to study the process of development of liver involvement in exotoxicosis.

MATERIALS AND METHODS

The study was conducted on 16 rabbits of the Chinchilla breed in 4 groups (in each 4 rabbits) weighing 3.0-3.5 kg.

The first group was intact and control animals. The remaining 12 rabbits were interrupted in a special chamber, they breathed with hydrochloric acid vapor during 7 days for 30 minutes.

On the 10th day, 4 rabbits (the second group) were taken out of experiment. The remaining 8 rabbits, beginning the 21st day of the experiment for 4 days, again breathed with vapors of hydrochloric acid. On the 30th day another 4 rabbits (3 group) were taken out of the experiment.

The 4th group of rabbits, beginning on the 50th day of the experiment for 4 days, again breathed with vapors of hydrochloric acid and 60 days were taken out of experiment.

On the 10th, 30th and 60th day of the experiment, the concentration of medium molecular weight peptides (MMWP), the amount of total bilirubin and creatinine, as well as the level of thymol in the blood taken from ear veins of the rabbit, were determined in intact animals.

The concentration of medium molecular weight peptides (MMWP) was determined by the method of V.S.Kaminshikov, the amount of total bilirubin and creatinine and the level of thymol by using the reactive sets manufactured by Human - Bioscreen MS-2000 microanalyzer.

The obtained quantitative indices were statically processed by a nonparametric method - the Wilcoxon (U) test (Manna-Witni).

The calculation was carried out using the EXEL electronic table compiled in the AMU.

In carrying out the experiment on animals, the rules 86/09 EEC of the adopted Bioethical League of Europe and UNESCO (Paris) were strictly observed.

RESULTS AND DISCUSSION

The concentration of medium-molecular peptides in the blood obtained in intact animals was varied in the range 0.23-0.26 cu. On average, the concentration was 0.243 ± 0.003 cu.

The total amount of bilirubin was 7.7-20.5 $\mu\text{mol/l}$, creatinine 58.4-78.1 $\mu\text{mol/l}$, and thymol level 1-5. The average arithmetic parameters were 13.9 ± 1.2 $\mu\text{mol/l}$, 67.8 ± 1.8 $\mu\text{mol/l}$, and 2.69 ± 0.36 , respectively.

The results were accepted as a norm and were compared with the results obtained from experimental animals.

In the second group of experimental animals, after 3 days of cessation inhalation with hydrochloric acid vapor, the concentration of MMP in the blood was 8% ($p < 0.001$) and the mean score was 0.263 ± 0.003 cu.

In contrast to the concentration of MMP, the increase in the level of total bilirubin was higher. Since, on the 10th day of the experiment, the average level of bilirubin in the blood ($M \pm m = 26.1 \pm 0.8$), in comparison with the first group, increased 87% or 1.9 times.

The amount of creatinine in the blood compared to the control group increased 25% or 1.3 times ($p < 0.001$) and reached 84.9 ± 1.3 $\mu\text{mol/l}$. As a result, the level of thymol, which characterizes the toxic state of the body, increased sharply. The level of thymol in comparison with the first group is increased 95% or 1.9 times ($p < 0.001$) ($M \pm m = 5.17 \pm 0.63$ $\mu\text{mol/l}$).

Thus, in rabbits breathing hydrochloric acid vapor for 7 days, according to the indicators of intoxication (total bilirubin, creatinine and thymol), the concentration of MMP, which is a characteristic marker of inflammation, is increased.

On the 30th day of the experiment, the concentration of MMP continued to increase in the blood of the 3rd group of animals. The average concentration was 0.268 ± 0.003 cu. Compared with the first group, this indicator is increased 10% ($p < 0.001$), compared to the second group only 2%.

It is clearly seen that as the number of breaths increases with hydrochloric acid vapor, the process of inflammation becomes more organized.

Despite a 14 day break between the first and second 7 day inhalation with hydrochloric acid vapor, breathing with hydrochloric acid vapor creates

a second wave of inflammation. Therefore, the developed pathological process becomes chronic.

On the 30th day of the experiment, in addition to increasing the MMP in the blood, and increases the amount of total bilirubin. Its average level, in comparison with intact animals, is increased 114% or 2.1 times ($p < 0.001$) and is reached to 29.8 ± 1.0 $\mu\text{mol/l}$. In comparison with the second group, the growth was not so high, it was only 14%. The results show that, in animals inhaled with hydrochloric acid vapor, the detoxification function of rabbits was severely impaired.

Also, the amount of creatinine in the blood is increased significantly. This increase in comparison with intact animals is increased 39% or 1.9 times ($p < 0.001$). And in comparison with the second group, this indicator is increased 11%.

Thus, in the blood of rabbits, inhaled with hydrochloric acid vapor (on the 30th day of the experiment), the average amount of creatinine is increased 94.4 ± 1.7 $\mu\text{mol/l}$. The obtained data show the transition of the inflammatory process to the kidneys.

The level of thymol, characteristic for general intoxication process, continued to increase. The mean quantitative index of thymol on the 30th day of the experiment, compared with intact animals, increased 2.9 times or 193% ($p < 0.001$) and became 7.88 ± 0.67 , and in comparison with the second group, the level of thymol also is increased. But by comparing with intact, this increase was 52%.

Continued increase in the level of thymol in the blood indicates the involvement of the body in general intoxication.

On the 60th day of the experiment, the mean concentration of MMP in the blood continued to increase. This increase, in comparison with intact group was 14%, compared with the second group 5%, and in comparison with the third group 3%.

Table. The dynamics of changes in inflammation and intoxication markers in the blood of rabbits breathing with HCL steam

№8	Marker	Statistik information	Intact station n=16	Time of investigation		
				10 days n=12	30 days n=8	60 days n=4
1.	MMP	Min	0.23	0.244	0.258	0.270
		Max	0.26	0.278	0.283	0.284
		min±max	0.243 ± 0.003	0.263 ± 0.003	0.243 ± 0.003	0.277 ± 0.003
2.	Common bilirubin	Min	7.7	21.0	25.0	29.0
		Max	20.5	30.2	33.6	35.0
		min±max	13.9 ± 1.2	0.261 ± 0.008	29.8 ± 1.0	32.8 ± 1.3
3.	Kreatinin	Min	58.4	78.6	88.6	92.7
		Max	78.1	91.4	100.0	105.6
		min±max	67.8 ± 1.8	84.9 ± 1.3	94.4 ± 1.7	100.8 ± 2.9
4.	Timol	Min	1	2	5	9
		Max	5	9	11	12
		min±max	2.69 ± 0.36	5.17 ± 0.63	7.88 ± 6.67	10.5 ± 0.65

Note: In all cases $P < 0,001$

In the fourth group of animals inhaled with hydrochloric acid vapor for the third time, the inflammatory process continued to develop.

In the blood taken from the fourth group of animals, the amount of total bilirubin is changed sharply, and it became $32.8 \pm 1.3 \mu\text{mol/l}$. In comparison of the first group, this indicator is increased 2.3 times or 134.9%, compared to the second group of 26%, compared to the third group of 10%.

The average amount of creatinine was $100 \pm 2.9 \mu\text{mol/l}$, and compared to the first group, 49% ($p < 0.001$), comparing the second group 19%, and comparing the third group 7%.

It should be noted that under the influence of hydrochloric acid vapors, the level of creatinine compared with bilirubin moderately increased. But, the inhalation with hydrochloric acid vapor sharply affects the level of creatinine and this effect becomes more pronounced on the 60th day of the experiment (table).

As can be seen, in rabbits inhaled vapors of hydrochloric acid, the antitoxic function of the liver and kidneys was disrupted.

On the 60th day of the experiment, in the blood of the fourth group of animals, the average level of thymol is reached the highest figure, and became 10.50 ± 0.65 . This increase, in comparison with intact groups, is increased 3.9 times or 291% ($p < 0.001$). Comparisons with other groups show continued increases in thymol levels. Since, the level of thymol, on the 60th day of the experiment, in comparison with the second group 103% and in comparison of the third group 33% is increased, (table).

Thus, according to the results of our research, it can be concluded that the inflammatory process that developed as a result of exogenous intoxication of the organism creates a focus of endogenous intoxication, and the development of a pathological process leads to toxicity.

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Toksikozun Müddətindən Asılı Olaraq İltihab Markerlərinin Dəyişmə Dinamikası

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Azərbaycan Tibb Universitetinin Elmi-Tədqiqat Mərkəzi

Tədqiqatın əsas məqsədi ekzotoksikoz zamanı iltihab prosesinin inkişaf dinamikasını izləmək olmuşdur. Bu məqsədlə təcrübələr 16 baş şinşilla cinsinə mənsub olan dovşanlar üzərində aparılmışdır. Onlar 4 qrupa bölünmüşdür. Ekzogen intoksikasiya modeli: təcrübə heyvanları xüsusi hazırlanmış kameralarda HCL buxarı ilə tənəffüs etdirməklə yaradılmışdır. Təcrübə heyvanlarından götürülən qanda orta molekullu pepidlərin, ümumi bilirubin, keratinin və timolun miqdarını təyin etməklə ekzogen intoksikasiyanın orqanizmdə yaratdığı patoloji prosesin patogenezinin bəzi məqamları aydınlaşdırılmışdır. Müəyyən edilmişdir ki, ekzogen intoksikasiya orqanizmdə ilk əvvəl iltihab ocağı yaradır və orada yaranmış toksiki maddələr hesabına endogen intoksikasiya keçid alır.

Açar sözlər: Toksikoz, ekzogen intoksikasiya, total bilirubin, kreatin

Изменение Динамики Маркеров Воспаления В Зависимости От Времени Токсикоза

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Главной целью исследования являлось изучение изменения динамики воспаления при экзотоксикозе. Исследование проводилось на 16-ти зайцах породы шиншилла. Моделирование экзотоксикоза проводилось в особой камере путем ингаляции парами HCl. Взятая у животных экспериментальной группы кровь была исследована на содержание пептидных молекул, общего билирубина, креатинина и количества тимола. Было выявлено, что экзогенная интоксикация, в первую очередь, создает очаги воспаления в организме, а потом этот процесс переходит в эндогенную интоксикацию.

Ключевые слова: Токсикоз, экзогенная интоксикация, тотальный билирубин, креатин

Mycological Assessment Of Woody Plants Used For Greening In Baku

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As a result of the conducted studies, it has been established that 95 species of fungi participate in the formation of mycobiota of more than 100 tree species used in the greening of the city of Baku. From them 5 species (*Herpotrichia juniperi* (Duby) Petr., *Cenangium abietis* (Pers.) Duby, *Dothidella ulmi* (C.-J. Duval) G. Winter, *Fusicladium saliciperduum* (Allesch. & Tubeuf) Tubeuf, *Onnia triqueter* (Pers.) Imazeki) are new for the mycobiota of Azerbaijan and 69 species for pathogens. It was established that the trees like as Ordinary pine, Eldar pine, juniper, cypress, Japanese medlar, stone oak, oriental plane and others were more resistant to the impact of pathogens and their use in the greening of the Baku city is advisable.

Keywords: Planting of greenery, mycobiota, phytopathogenic species, prevalence frequency, resistant species

INTRODUCTION

As is known, greenness and planting of greenery as an integral structure of urbanization are constant a component of ecological carcass. So that, they able to perform like as environment-forming and protecting factor also as a valuable surveillance and control object to protects the comfort life of people, and as an indicator of conditions and quality of life in the any residential area (Avdeeva, 2008). In other words, they do not only beautify of the city, they also has important ecological function like as sanitary-hygienic, recreation, historical, environment forming and others.

On the other side, the growth conditions of plants which forms the greenness in the urban environments sharply differs from the same plants in the natural environment by ecological indicators. It is also causes to change the condition of plants which is using in the planting of greenery (Kovyazin et al., 2002). As a result, in some of them is observed unpleasant situations like as plant leaves drying up, gradually losing their decorative appearance and biological activity and others. Although, the cause of these cases is different, the role of microorganisms, primarily role of fungi which causes pathologies is not in the last place. Because as a result of fungi activity plants could be weaken, even massively destroyed (Horst, 2013). In the formation of any region's biodiversity, also in the carrying out of planting of greenery are used both native and introductory plants (Korpauchinsky et al., 2013; Tomoshevich et al., 2013). Fungi and their pathogenic species is a natural contaminate of plants

and introduced plants cause to forms a new ecological environment for them, for that there is no doubt that the plants which used for this purpose is a potential source for the spread of pathogen species.

In the most of the work which carried out general in Azerbaijan and in the big cities (Baku, Ganja, Sumgayit, Shirvan, etc.) was dedicated to the taxonomic definition of pathogenic mycobiota, mainly to micromycet composition in the tree plants (Abdullayeva et al., 2014; Jabrayilzade et al., 2014). In most cases researchers content with observations to the pathogens as a fragment forms. From this point of view, is very important to assessment potential damage of plants cause by diseases and implementation of complex measures to combat pathogens, including investigation of additional sources in concrete ecological-geographical conditions, the biological analysis of parasitic fungi and the formation of pathocomplex, also determination the resistance of used plants to the effect of pathogens.

Therefore, the main purpose of the present work is to evaluate the taxonomic composition of the mycobitya of trees (aborigene and introduced) used for greening in Baku, the largest city of Azerbaijan and the influence of pathogens involved in its formation to the durability of the trees used for greening.

MATERIALS AND METHODS

Samples for researches were taken from trees in streets and avenues, parks and gardens and these areas were mainly located in the central part of the city of Baku (located in some areas of the Sabail,

Yasamal, Nasimi, Narimanov, Khatai Sabunchu and Surakhani districts of Baku) which has anthropogenic burden.

The samples were taken systematically (Route) and unsystemically, and the taken samples were analyzed according by the known mycology methods (Methods of experimental mycology, 1982). Samples also were taken in the different seasons. Overall, during the period of research were taken approximately 1000 samples.

The frequency of use of plants in urban greening is calculated according to the following formula:

$$N = n/s,$$

here N - using frequency (pcs / ha), N - the number of concrete species found in the studied street, avenue, park, and etc. places.

The frequency distribution of fungi on the trees used for greening, also spread rate of diseases caused by pathogenic cultures were calculated according to the following formula:

$$P=(n/N) \times 100$$

here, P - frequency rate of fungi according to the sample (or spread rate of diseases caused by pathogenic-%), n - number of founds fungi (the total number of infected plants in the study area, piece), N - the total number of samples (Total number of plant species taken from sample).

RESULTS AND DISCUSSION

As noted, the purpose of the presented work is devoted to the researches related to the micrological evaluation of plants used in greening of Baku city. For this reason in our research firstly were considered appropriate to characterize the species composition of fungi which resides in these plants, more accurate in this trees. Before bringing the information about the taxonomic structure of the recorded fungi, we would like to bring some statistical information about of trees which used for greening. According to the literature and to the our observations in Baku the number of local and introduced trees which is using in the greening more than 100 species and their used is characterized by different rate in the urban greening (Abdullayeva et al., 2014). Thus, among of broad-leaved which is used in the greening of Baku city is encountered plants like as European olives, Japanese sakura, East willow, but among of coniferous is encountered plants like as Eldar pine

and cypress. At least for the reason that, this plants is encountered in any streets, parks and gardens. In addition, poplar, white mulberry and etc. plants also considered widely used in the greening of Baku.

As a result of analysis of samples taken from these or other trees, it became clear that in the formation of overall mycobiota of this trees participates 95 species of fungi. Their taxonomic structure was given in the table 1. according to the system which given on the official website of the International Association of Micology (<http://www.mycobank.org/MycoTaxo.aspx>).

As seen, the number of anamorphs of sac fungi are much more than from the fungi belonging to the other taxonomic groups and 50,5% of fungi recorded during research fall to their share. The second place with 31.6% belongs to Basidial fungi, the next places with 10,5% belong to anamorphs of sac fungi, and 7,4% to the Zygomycetes. It should be noted that, many fungi were recorded in the various mycological research in different biotope of Azerbaijan, that is, most of their areal is included the territory of Azerbaijan. However, among of registered fungi met fungi which was firstly recorded in the territory of Azerbaijan. Their taxonomic affiliation and information about the plant- owner was given in the table 2. As seen, 3 of 5 types that fitting this characteristic belong to the telemorphs of sac fungi, 1-to the anamorphs, 1 to the basidial fungi. Interestingly that, all of these fungi recorded for the first time are related to a certain extent due to pathogenicity and cause different diseases in their own plants. One of the interesting aspects of the issue is that, this fungi were recorded in the plants which mainly used in the greening of Baku in the recent years. This fact allows to record the importance of improvement of the monitoring system for the carefully checked of plants used in the greening in terms of mycological safety.

Although this information may have a general view but it can not be considered sufficient for the evaluation of the mycological safety of trees used in the greening in the city of Baku. For this reason, at the next stage of the research was identified pathologies caused by registered fungi, the distribution of fungi and their pathologies on the different species of trees and trees resistant to pathologies.

Table 1. Taxonomic structure of fungi located in the trees which used in the greening of Baku city.

Kingdom	Division	Class	Order	Family	Genus	Species
Mycota	Zygomycota	1	1	1	3	7
	Ascomycota (Telemorf)	1	3	3	6	10
	Ascomycota (Anamorf)	4	8	11	25	48
	Bazidiomycota	2	5	9	19	30
	Total	8	16	23	46	95

Table 2. Taxonomic affiliation of fungi species which first time were registered in the Azerbaijani nature.

Division	Species	Own plants
Ascomycota (T)	<i>Herpotrichia juniperi</i> (Duby) Petr.	Juniper
Ascomycota (A)	<i>Cenangium abietis</i> (Pers.) Duby	Fir-tree
	<i>Dothidella ulmi</i> (C.-J. Duval) G. Winter	Fir-tree
	<i>Fusicladium saliciperdu</i> (Allesch. & Tubeuf) Tubeuf,	Ulmus
Bazidiomycota (B)	<i>Onnia triqueter</i> (Pers.) Imazeki	Willow
		Fir-tree

Table 3. Specific weight of pathogens among registered fungi.

Division	Total number of registered fungi species	Which were included to the epiphyte mycobiota or do not have pathogenicity	Species which have confirmed of pathogenicity	The status of those who unknown
Zygomycota	7	5	1	1
Ascomycota - T	10	1	8	1
Ascomycota - A	48	7	37	4
Bazidiomycota	30	5	23	2
Total	95	18	69	8

Table 4. Distribution of registered fungi on the separate trees used in the greening in the Baku city.

Plants	Zygomycota	Ascomycota	Bazidiomycota	Total number of registered species	Spread rate of pathogens(%)
East willow	1	7	5	13	3,4
European olives	1	19	3	23	7,6
Japanese safura	1	17	4	22	56,7
Eldar pine	0	6	2	8	0,5
Ordinary poplar	1	19	12	32	33,4
White mulberry	1	15	4	20	31,7
Chinese Ailanthus	1	13	3	17	12,1
Ordinary cypress	1	5	2	8	0,9
Japanese medlar	0	9	1	10	0,8
Southern willow	1	18	14	33	34,5
Stone oak	0	8	4	12	2,1
White acacia	1	18	8	27	19,6
Ulmus	1	12	6	19	9,8
Ash-tree	0	14	6	20	7,6
Others	1	20	6	30	0,2-7,6

It is known that in the formation of pathology are involved one or more types of fungi (Horst, 2013). According to the literature data, also to the our observations were identified that most of the recorded fungi are related to one or more pathogenicity (Table 3). As seen, 14.7% of the registered fungi belongs to the saprotrophs which are fed only with dried plant organs, they also participate in the formation of the epiphythmic mycobiota of these plants. As seen, the remaining fungi by ecolo-trophic view belongs to the biotrophs or polytrophs. Logically, all these can be characterized as a pathogenic related species, but it can not be considered sufficient to say this idea about the results which obtained from our or other researchers. For this reason, we considered necessary to combine some fungi in a third group, namely in the unidentified group. 8,4% of fungi recorded in our research is corresponds to the this characteristic which to determination of their status can be identified as a result of future research.

The next issue of research were to identify the distribution of pathogenic species of fungi which

participating in the formation of mycobiota of different types of trees used in the greening of Baku city. It became clear that, the mycobiota of plants used in greening differ from each other according to the species composition (Table 4). As seen, by the number of recorded fungi Southern willow and Ordinary poplar is in the first place, but for the degree of spread rate of pathogens Japanese safura is in the first place.

Interestingly, most of pathogens that spread on the plant are related to one species, ie *Inonotus hispidus*. We can say, these fungi is encountered in one of the two plant in Baku city. Although, is encountered the ephitotia associated with the spread of the fungi but, during the research, as well as the analysis of literature data, did not detect full dryness of these plants due to effect of this fungi. This situation also can be characterized as mild parasitism.

It would be good to touch one issue, about greening of Baku city, namely trees which used for the greening in the parks and gardens. This is due to the fact that Baku is located in the Absheron

Peninsula, one of the most drought regions of Azerbaijan, as well as in the Caucasus and therefore, here subtropical plants is more and their weight in urban greening is quite high (<http://eco.gov.az/en>). The results about these plants namely about olive are presented in Table 4, but information about of mycobyta of other dry subtropical plants, such as almond, pistachio, ziziphus, elaeagnus, etc. is presented in the generalized forms in the same table under "others". Among the fungus involved in the formation of the mycobiota of these dry subtropical plants, are encountered representatives of all taxonomic structures of the Mycota kingdom and inside these plants to impact of fungi most durable was ziziphus, and most unstable was pistachio. Nevertheless, a number of issues (Influence of fungi-plant relationships to their biological productivity and to the decorative appearance and the issues related to explanation of character of changes for that reason) related to the use of these plants in the conditions of Absheron, especially in the creation greening areas have not been solved scientifically, so the issue is open to research.

At the end of the research were conducted experiments to obtained information about diseases

caused by pathogenic fungi participating in the formation of mycobiota of trees used for greening in the Baku city, their prevalence rate and information about developmental cycles of some of the disease-makers fungi. It became clear that, the most spreads disease is brown decay and leaf stains which in their occurrence are participate various types of fungi (Table 5). As seen, the most commonly spread diseases were on the plants like as Japanese falcon, southern willow, ordinary poplar, white mulberry, and so on. In generaly, it should be noted that the plants used in the greening in the Baku city shows different resistance to diseases caused by fungi which allowed us to notes the advisability to characterize these trees in this aspect and in this aspect distribution rate of diseases was taken as the main distinguishing criterion.

It became clear that, the trees used for greening in Baku in general can be divided into five groups (Table 6). As seen, into first group includes highly resistant plants like as conifers plants and species that are relatively new in the history of use in greening, in them distribution rate of common diseases is not more than 5%.

Table 5. Fungal diseases observed in the trees used in the greening in the Baku city and their prevalence rate (%).

N	The name of the disease	Disease causing fungi	Observed tree	Distribution rate
1	Brown trunk decay	<i>I.hispidus</i>	Japanese safura White mulberry White acacia	12,6-56,7
2	Brown trunk decay	<i>F.pinicola</i>	Ordinary poplar South willow	7,8-12,8
	Brown root decay	<i>F.pini</i>	Eldar pine Ordinary pine	2,1-4,3
3	White trunk decay	<i>F.fomentarius</i> <i>G.applanatum</i>	Ordinary poplar South willow	7,8-11,2
4	Stains	<i>A.alternata</i> <i>C.microsora</i> <i>L.fumago</i> <i>Ph.opuli</i> <i>S.populi</i> <i>Ph.ulmi</i> <i>Rh.salicinum</i>	European olives Linden South willow Stone oak Ulmus	1,2-3,9
5	Rust	<i>M.populnea</i> <i>M.salicina</i>	South willow Ordinary poplar	1,7-3,4
6	Powdery mildew	<i>E.alphitoides</i>	European olives South willow	2,3-5,4
7	Necrosis	<i>N.cinnabarina</i> <i>C.quercina</i>	Ordinary poplar Ulmus Linden Stone oak	0,7-1,4

Table 6. Characteristics of trees used in the greening in the Baku city according to the sustainability to the diseases.

Species having high durability	Durability species	Slightly disease-carrying	Medium degree disease-carrying	Strongly disease-carrying
Ordinary pine Juniper Cypress Japanese medlar	Stone oak Plane Eldar pine Ordinary eucalyptus	Gleditsia caspica Chinese Ailanthus Fraxinus Crying Willow	White acacia Ulmus Linden European olives	Japanese safura South willow Ordinary poplar White mulberry

In the secondary group includes durably trees species like as coniferous and broad-leaved trees and in them distribution rate of diseases is up to 10%. In the next places are includes those weaker than 20%, 30% and over than 30%. In our view, is useful to take them into account in the greening to provide the anthropogenic environments on the view of Sanitary-hygienically.

From the carried out of research was determined that, the trees used in the greening of Baku city are characterized by rich mycobiota and in the formation of mycobiota are participate 95 species of fungi. From them 5 species are new for the mycobiota of Azerbaijan. The sustainability indicator of used trees on pathogens is different. To used the trees like as Ordinary pine, cypress, Japanese medlar, stone oak, oriental plane and others in the greening of Baku city is more advisable.

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Bakı Şəhərinin Yaşıllaşdırılmasında İstifadə Edilən Oduncaqlı Bitkilərin Mikoloji Qiymətləndirilməsi

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Aparılan tədqiqatlardan müəyyən edilmişdir ki, Bakı şəhərinin yaşıllaşdırılmasında istifadə edilən 100 növdən artıq ağacın mikobiotasının formalaşmasında 95 göbələk növü iştirak edir. Qeydə alınan göbələklərin 5 növü (*Herpotrichia juniperi* (Duby) Petr., *Cenangium abietis* (Pers.) Duby, *Dothidella ulmi* (C.-J. Duval) G. Winter, *Fusicladium saliciperduum* (Allesch. & Tubeuf) Tubeuf, *Onnia triqueter* (Pers.) Imazeki) Azərbaycan təbiətinə xas olan mikobiota üçün yeni, 69 növ isə patogenlərə aiddir. İstifadə edilən ağacların patogenlərin təsirinə davamlılıq göstəricisinin qiymətləndirilməsi nəticəsində müəyyən edilmişdir ki, Bakı şəhərinin yaşıllaşdırılmasında Adi şam, Eldar şamı, Ardic, Sərv, Yapon əzgili, Daş palıd, Şərq çınarı və s. kimi bitkilərin istifadəsi daha məqsəduygundur.

Açar sözlər: Yaşıllaşdırma, mikobiota, fitopatogen növlər, yayılma dərəcəsi, davamlı növlər

Микологическая Оценка Древесных Растений, Используемых В Озеленении Города Баку

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В результате проведенных исследований установлено, что в формировании микобиоты более чем 100 видов деревьев, используемых в озеленении города Баку, участвуют 95 видов грибов. Обнаруженные 5 видов грибов (*Herpotrichia juniperi* (Duby) Petr., *Cenangium abietis* (Pers.) Duby, *Dothidella ulmi* (C.-J. Duval) G. Winter, *Fusicladium saliciperduum* (Allesch. & Tubeuf) Tubeuf, *Onnia triqueter* (Pers.) Imazeki) оказались новыми для микобиоты Азербайджана, а 69 видов – патогенами. При оценке показателя устойчивости используемых деревьев к действию патогенов выявлено, что в озеленении города Баку наиболее целесообразно использование таких растений как сосна обыкновенная, Эльдарская сосна, можжевельник, кипарис, мушмула японская, дуб каменный, восточный чинар и т.д..

Ключевые слова: Озеленение, микобиота, фитопатогенные виды, степень распространения, устойчивые виды

Dendroclimatic Investigation of the Chestnut-Leaved Oak (*Quercus castaneifolia* C.A.Mey.) Distributed in Lerik Region of Azerbaijan

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Climate change and extreme natural events have a negative impact on the growth of most plants. The purpose of this study is to investigate the effects of the environmental factors on the radial growth of the chestnut leaved oak spread out in the territory of Lerik region. It was revealed that the *Quercus castaneifolia* C.A.Mey., which are spread in this area, are very sensitive to changing of environmental conditions. The plant negatively reacted to the short-term temperature drop and in the result formed frost rings.

Keywords: Climate change, radial growth, Lerik, *Quercus castaneifolia*, dendroclimatic investigation, anatomical methods.

INTRODUCTION

In the nature, most of the trees are very sensitive to the changes of hydrological regime, temperature and light conditions. Often these changes are being instantly, short term or long term. In these times in trees occurring catastrophic or small damages in the structure of the trees. Plants growing in different environments, altitudes and conditions are reacting to this kind of changes by following way: tree migration, leave fall or damages in alive cells (Schweingruber, 2007; Stockli and Schweingruber, 1996). Trees reaction to the changing conditions allow us to determine distribution limits of the adapted trees.

Dendrochronology is one of the most important environmental techniques for a variety of natural environmental processes and a monitor for human caused changes to the environment such as pollution and contamination. Dendrochronology examines events through time that are recorded in the tree-ring structure or can be dated by tree-rings. Because tree becomes the instrument for environmental monitoring, it serves as a long-term bioindicator that extends for the lifetime of the tree. Trees record any environmental factor that directly or indirectly limits a process that affects the growth of ring structures from one season to the next, making them usefully monitor for a variety of events.

Extremal climate conditions like long-term droughts and short-term frosts negatively affecting radial growth of the trees. With application of the dendroclimatic methods and analyzing of scars and damages in the stem of tree it is possible to determine the past climatic conditions. Generally for studying past climate are using tree-ring width. Only in few cases analyzing anatomical structure of the steam. During extreme events, in the tree rings occurring traumatic resin ducts, compression and tension tree

rings, callus rings, frost rings. During all these events there observing abnormal cells like collapsed cells, callus cells and bent rays (Glock and et al., 1963; Studhalter, 1955). Drought rings anatomically belonging to the same category (Larson, 1995). We can observe these in the investigations of the some researchers (Stockli and Schweingruber, 1996).

Oak species is one of the most distributed and used for dendrochronological and for anatomical investigation in the world. There were distributed 450 species in the world, 19 species in the Caucasus and 9 species in Azerbaijan. *Quercus castaneifolia* is of the oak species, which mainly distributed in Hirkan forests (Сафаров, 1962).

In this study we used dendroclimatic and anatomical methods for analyzing impact of climate on the radial growth of the chestnut-leaved oak.

MATERIALS AND METHODS

The chestnut-leaved oak (*Quercus castaneifolia*) is native to the Azerbaijan and Alborz mountains of Iran. It is a deciduous tree growing up to 35 m tall, with a trunk up to 2.5 m diameter (exceptionally up to 50 m tall with a trunk up to 3.5 m diameter) (Prilipko, 1961). The leaves are 10–20 cm long and 3–5 cm wide, with 10–15 small, regular triangular lobes on each side (Fig. 1.). The flowers are wind-pollinated catkins; the fruit is an acorn, maturing about 18 months after pollination, 2–3 cm long and 1.5–2.0 cm broad, bicoloured with an orange basal half grading to a green-brown tip; the acorn cup is 2 cm deep, densely covered in soft 4–8 mm long 'mossy' bristles. The acorns are very bitter, but are eaten by jays and pigeons; squirrels usually only eat them when other food sources have run out (Prilipko, 1961; Tutayug, 1965).



Figure 1. View chestnut-leaved oak in study site.

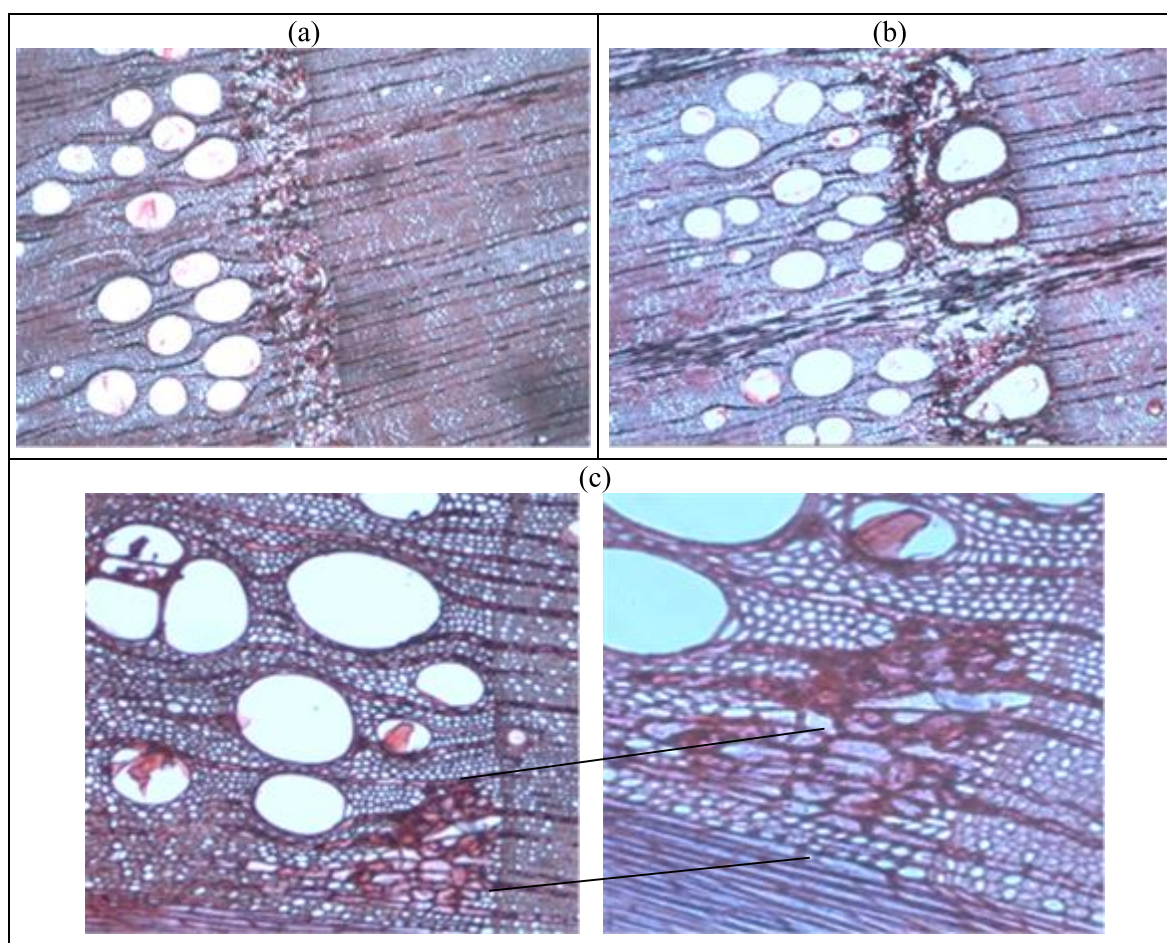


Figure 2. Early frost ring (a), late frost ring (b) and tongue like damages (c), which was observed in our samples.

We carried out investigations in Lerik region 1500 m a.s.l., which was located in 38°45'04'' N and 48° 22'36'' E geographical coordinates. This site is high limit of the distribution of *Quercus castaneafolia*. Samples was taken with increment and trephor borers. We took tree-ring core samples from 15 trees. Samples was prepared in the laboratory. For studying climate-growth relations was used dendroclimatic methods and anatomical structure was analyzed under microscope.

Frosts rings are anatomically abnormal, eco-physiologically pathological structures. Frost rings consisting of from thin wall-damaged tracheids, bent rays, callus cells, and morphologically reversed tracheids. The frost rings formed during the vegetation period have different anatomical structures. They can consist of phenol-prone single-cell sequences, gradually scattered, and then gradually expanding tracheids, from complete "frost rings" and small resin ducts.

RESULTS AND DISCUSSION

As mentioned before generally, the main cause of forming frost rings is the short-term drop of temperature. At the beginning of the vegetation period, the plant begins to operate actively. In broadleaf trees, vessels and fibers are responsible for water transportation. During temperature drop water are freezing in the vessels and fibers. Water freezing are destroying cell walls and disturbing the form of the cells completely or partially. In the result, forming frost rings or wounds.

We observed frost rings in our tree-ring samples (Fig. 2). Tree-ring core samples covered 1967-2012 years interval. For analyzing frost ring, we divided these years into two period: 1967-1989 and 1990-2012 years intervals. During our investigations, in the first period we observed 2 (1981, 1987), but in the second period 5 (1990, 1993, 2004, 2009, 2010) frost rings. Our analysis showed that latest years frequency of the frost rings are increased. This is sign of the new local growing conditions, which related with climate change. In our samples, we observed early, late frost rings and tongue like damages.

Bend cells are observing in all disturbances. The severe frost occurring during the beginning of the vegetation period and damaging first cell are called early frost ring, but frosts occurring later forming late frost rings. In spite of that, different tree species have different anatomy and frequency of frost rings, generally they have common features,

In Europe, frost rings forming rarely, even one time in 100 year or more late. It shows that trees adapted to extremal environmental conditions and can easily react them. However, due to the climate change, there forming new environmental conditions, which trees cannot react to the new extreme conditions by proper way and forming frost rings.

In our samples mean sensitivity coefficient was 0.305 and max value 0.384. Observed high confidents of sensitivity indicating that trees growing in this area is very sensitive to the changing of the local climate and they reacting to year-to year

climate variation. That is why during early spring when temperature is dropping more than usual, there forming frost rings in the steam of the tree.

The investigation of such damages has great importance. By studying these, it is possible to learn the effects of extreme climatic conditions on plants and to evaluate climate changes.

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**Azərbaycanın Lerik Rayonunda Yayılmış Şabalıdyarpaq
Palıdın (*Quercus castaneifolia* C.A.Mey.) Dendroiqlim Tədqiqi**

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İqlim dəyişkənliyi və ekstremal təbii hadisələr əksər bitkilərin artımına mənfi təsir göstərir. Bu tədqiqatda məqsəd mühit amillərinin Lerik rayonunun ərazisində yayılan şabalıdyarpaq palıdın radial artımına təsirini öyrənməkdən ibarətdir. Məlum olmuşdur ki, bu ərazidə yayılan şabalıdyarpaq palıdlar mühit amillərinin dəyişilməsinə çox həssasdırlar. Temperaturun qısamüddətli normadan aşağı düşməsi bitkinin artımına mənfi təsir göstərmiş və nəticədə oduncaqda don yaraları yaranmışdır.

Açar sözlər: *İqlim dəyişkənliyi, radial artım, Lerik, Quercus castaneifolia, dendroiqlim tədqiqat, anatomik metodlar.*

**Дендроклиматическое Исследование Каштанолистного Дуба
(*Quercus castaneifolia* C.A.Mey.) в Лерикском Районе Азербайджана**

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Изменение климата и экстремальные природные явления негативно влияют на прирост большинства растений. Целью этого исследования является изучение влияния окружающих факторов на радиальный прирост *Quercus castaneifolia*. Было выявлено, что каштанолистные дубы, которые распространены в этой области, очень чувствительны к изменяющимся условиям окружающей среды. Кратковременное падение температуры ниже нормы отрицательно сказывается на росте растения и способствует образованию на древесине морозобойных колец.

Ключевые слова: *Климатические изменения, радиальный прирост, Лерик, Quercus castaneifolia, дендроклиматическое исследование, анатомические методы*

The Characteristics of Flowering in *in situ* and *ex situ* Conditions of Species of *Pyrus* L. Genus on North-Eastern Part of the Greater Caucasus

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This article represents the comparatively research results the characteristics of flowering in cultural and natural conditions of 5 wild pears species which were spreading in northeastern part of the Greater Caucasus. As a result of investigation there has been identified that, species of pear studied on reproductive phase are normally flowering and producing fruit in cultural and natural conditions and the narrowing of their areal are based on anthropogenic factors than their biological characteristics.

Keywords: Greater Caucasus, northeastern, pear, reproductive, flowering, *in situ*, *ex situ*, ontogenesis

INTRODUCTION

The reproductive phase is one of the most important stages ontogenesis of all lively systems. It is known that the processes of flowering are possess different characteristics in plants kingdom. In the investigation time are learnt the species of plants at the age of blooming, flowering, producing fruit and seed problems in cultural and natural conditions. The entering to flowering phase tree and bush plants are depend on their individual biological characteristics also their ecological factors of environment (Gurbanov, 2005; Iskender, 2010).

In the investigation area which were spreading species 5 wild pears are learnt the comparatively characteristics of flowering and producing fruit in cultural and natural conditions. Therefore which were spreading species of wild pear on north-eastern of Greater Caucasus in *in situ* and *ex situ* conditions of flowering and producing fruit characteristics the comparatively were carried about learning scientific-research works by us.

MATERIALS AND METHODS

The material of research was organized both of cultural and natural conditions which were spreading north-eastern part of Greater Caucasus about 5 species of *Pyrus* L. genus (*Pyrus caucasica* Fed., *P. communis* L., *P. georgia* Kuthath, *P. vsevolodii* Heideman, *P. salicifolia* Pall).

The essential aim of research work is consist of which were spreading in research area in natural and cultural flora the species of pear on comparatively flowering and producing fruit process learning of maintain genefond of plants defining role and giving analysis. The fulfilling of research work was used a number of methods. So, researching rare

species plants for learning in natural condition took the key methods by Zaychev, 1981; Beideman, 1979; Coper, 1985; Gurbanov, 2004 and etc.

RESULTS AND DISCUSSION

In the research time which was spreading on north-eastern part of Greater Caucasus about 5 species of *Pyrus* L genus were learned on comparatively in cultural and natural conditions. Besides was specified on both 2 conditions in same plants numbering and maturing time of flowering and producing fruit. It is known that researching plants are flowered and produced fruit at the age of 6-7 in cultural condition (*P. caucasica*, *P. vsevolodii*), and depend on from species at the age of 8-11 in natural condition (*P. georgia*, *P. salicifolia*). This process in the same of these plants both 2 conditions were observed at the age of 7-11.

The phenological observations were showed that are possess 9-21% high producing fruit in natural condition depends on from species in comparison with cultural condition. In the research time species of researching plants the process of flowering was learned on comparatively in natural and cultural conditions and was identified with time the beginning of flowering, ending, continuing of flowering period and life period of flower in every type (table 2).

In the research time in every species of plant were specified on both conditions numbering and maturing time of flowering and producing fruit. The phenological observations were showed that are possess high producing fruit in *in situ* condition depends on from species in comparison with *ex situ* condition.

Table 1. *In situ* and *ex situ* conditions of research plants flowering, producing fruit and seed.

№	Species	The age of flowering		Is flowering		Producing fruit and seed	
		<i>Ex situ</i>	<i>In situ</i>	<i>Ex situ</i>	<i>In situ</i>	<i>Ex situ</i>	<i>In situ</i>
1.	<i>Pyrus caucasica</i>	7	10	+	+	+	+
2.	<i>Pyrus communis</i>	6	9	+	+	+	+
3.	<i>Pyrus georgia</i>	7	9	+	+	+	+
4.	<i>Pyrus vsevolodii</i>	6	8	+	+	+	+
5.	<i>Pyrus salicifolia</i>	7	11	+	+	+	+

Table 2. The characteristics of flowering in cultural and natural condition the species of *Pyrus* L. genus

№	Species	<i>Ex situ</i>				<i>In situ</i>			
		Flowering				Flowering			
		Beginning	Ending	The period of cont. (day)	Life period of flower (day)	Beginning	Ending	The period of cont. (day)	Life period of flower (day)
1	<i>P. caucasica</i>	19.04	02.05	13	7-9	24.04	11.05	17	7-10
2	<i>P. communis</i>	18.04	25.04	11	8-9	25.04	10.05	15	9-11
3	<i>P. georgia</i>	16.04	27.04	13	8-10	28.04	13.05	15	8-11
4	<i>P. vsevolodii</i>	17.04	29.04	12	6-10	10.04	25.04	15	7-9
5	<i>P. salicifolia</i>	19.04	03.05	14	7-10	16.04	29.04	13	8-11

The result of phenological observations which carried out and information of literature were showed us researching plants belong to *Rosaceae* family entering reproductive phase is faster than tree and bush plants which belonging other families (Iskender, 2008; Iskenderov, Guliev, 1990; Costina, 2005).

It is known that carried out observations begins late between 3-4 years which are researching in *in situ* condition the species of *Pyrus* L. rather entering to reproductive phase in *ex situ* condition plant. The basic reason of this condition is growing and do not have agrotechnical service. The agrotechnical services of making in *ex situ* condition to plants are one of the accelerating factors of entering to productive phase.

It is known that it can affect living environment the factors of climate and structure of phytocenosis spreading the same species on this or that degree reaching to maturity age of plants (Guliev, Iskenderov, 1987; Lesica, Gurbewch, Crone, 2006).

The flowering characteristics which are learned plants in condition (ANAS, Central Botanical Garden) Absheron that had been introduced all of the species research about *Pyrus* L. genus entered the phase of flowering. The phonological observations were showed which carried out is observed flower fall down process before reproduction in species *P. salicifolia*. The reason of this we can explain that depending on from humid, hot and wind. We can say that the phenonological observations which carried out were not observed directly proportional continuing period of flowering between life periods of flower. As seen from here we can say that there is no dependence life period of

flower with flowering phase. We can connect it with individual biological characteristics of plant. It showed that comparatively analysis the process of flowering in *ex situ* and *in situ* the continuing period of flowering and life period of flower there is no difference between two conditions. In the researching time were observed that plants which are in *ex situ* condition the continuing period of flowering comparison with in *in situ* more 1-5 days. The happening of this difference mainly we can coordinate climate factors and also with the factor of temperature. It was identified that the result of analysis rare plants normally flowering and producing fruit.

Thus researches which carried out and the results of analysis has been identified that investigated plants normally flowering and producing fruit in every two conditions.

On this we can say that the narrowing area of these plants is depending on anthropogenic factors than their individual biological characteristics.

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Böyük Qafqazın Şimal-Şərq Hissəsində *Pyrus* L. Cinsi Növlərinin *in situ* və *ex situ* Şəraitlərində Çiçəkləmə Xüsusiyyətləri

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Məqalədə Böyük Qafqazın Şimal-Şərq hissəsində yayılmış 5 yabanı armud növünün çiçəkləmə xüsusiyyətləri mədəni və təbii şəraitlərdə müqayisəli şəkildə tədqiq edilmişdir. Tədqiqat nəticəsində müəyyən olunmuşdur ki, reproduktiv mərhələsi öyrənilən armud növləri mədəni və təbii şəraitlərində normal çiçəkləyib meyvə verir və həmin bitkilərin təbiətdə areallarının daralması onların bioloji xüsusiyyətlərindən çox antropogen amillərdən asılıdır.

Açar sözlər: Böyük Qafqaz, şimal-şərq, armud, reproduktiv, çiçəkləmə *in situ*, *ex situ*, ontogenez

Особенности Цветения В Северо-Восточной Части Большого Кавказа Видов Рода *Pyrus* L. В Условиях *in situ* и *ex situ*

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В статье проводится сравнительное исследование характеристики цветения в культуре и природных условиях 5 видов диких груш, распространенных в северо-восточной части Большого Кавказа. В результате исследования было выявлено, что груши, у которых изучался репродуктивный этап, нормально цветут и плодоносят как в условиях культуры, так и в природе. Уменьшение ареала этих растений в природе зависит в основном не от биологических условий, а от антропогенных факторов.

Ключевые слова: Большой Кавказ, северо-восток, груша, репродуктивные, цветущие *in situ*, *ex situ*, онтогенез

A Rare Case Report: The Cause of Painless and Gross Hematuria Is Primary Amyloidosis of the Bladder

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Primary amyloidosis of the urinary bladder is a rare disease, with only approximately 200 cases reported in the literature. Localized bladder amyloidosis is a rare disease that mimics neoplasia clinically, cystoscopically, and radiologically. The physiopathology is unknown, the prognosis is usually good and there is no specific treatment. Here we present a case report of the bladder amyloidosis, diagnosis and management of this rare condition.

Keywords: Amyloidosis, transurethral resection, hematuria

INTRODUCTION

Amyloidosis is characterized by extracellular deposits of the fibrillar protein, amyloid (Huang et al., 2006). It was first described by Virchow in 1853 (Virchow, 1854). Primary amyloidosis of the urinary bladder is a rare disease, with only approximately 200 cases reported in the literature. Bladder amyloidosis is considered to be a rare occurrence with the first case of primary bladder amyloidosis being described by Solomin in 1897 (Auge and Haluszka, 2000). Both sexes are equally affected between the fifth and seventh decade. Painless gross haematuria is the main presenting symptoms in most (>75%) cases (Johansson and Cohen, 1996). Localized urinary tract amyloidosis (UTA) is a rare disease that mimics neoplasia clinically, cystoscopically, and radiologically. Amyloidosis is categorized into two forms:

- Primary amyloidosis – The process in which plasma cells overproduce protein rich portions of antibodies known as light chains (AL), these proteins are then deposited within the viscera. This is a primary condition requiring no secondary influencing condition.
- Secondary amyloidosis (AA) – is most commonly associated with chronic inflammatory conditions such as rheumatoid arthritis, chronic osteomyelitis, or malignancies. Here, we see widespread systemic deposition of amyloid proteins.

CASE REPORT

A 45 year old female patient in July 2016, presented with history of intermittent episodes of gross total painless haematuria of 2 months duration. She had no known drug allergies. Surgical history included appendectomy. There was no significant

family history. She was a non-smoker. Laboratory examination revealed no significant abnormality. Sonography showed multiple solid lesion posterior wall of bladder suggestive of transitional cell carcinoma. Further evaluation with CT scan of abdomen and pelvis was done showing multiple small lesions in the posterior bladder wall without bladder wall infiltration to the adjacent structures. No systemic deposits were seen. No ascites was defined. At flexible cystoscopy with local anastesiawas seen a solid lesion on the posterior bladder wall resembling invasive bladder (Fig 1).

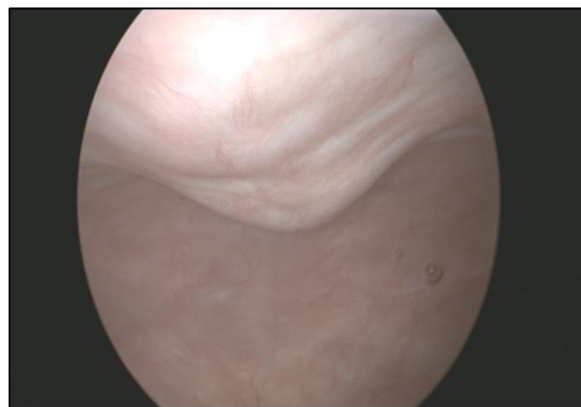


Figure 1. Cystoscopy image.

Transurethral resection was performed and submitted for histological examination. Macroscopic haematuria disappeared spontaneously after the treatment. Histopathology of the biopsied material showed no evidence of cancer.. Immunostaining of the biopsied material with Congo red stain confirmed the presence of amyloid fibrils in the biopsy material confirming the diagnosis of urinary bladder amyloidosis (Fig 2). The patient consulted to nephrology and gastroenterology for systemic screening. No evidence of systemic amyloidosis

was found. Serum protein electrophoresis showed non-specific abnormalities. Intravesical dimethyl sulfoxide (DMSO) for the 10 weeks and oral colchicine therapy was started. The patient has been followed up for 17 months and is currently free of symptoms. Cystoscopy was repeated every 3 months and did not show any recurrence of amyloid within a 17 month follow-up period.

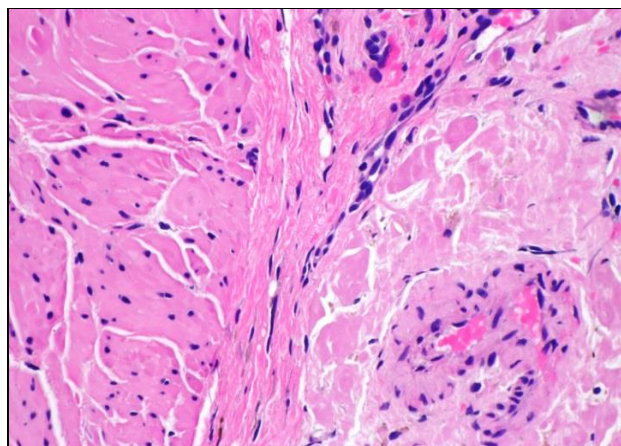


Figure 2. Apperance of nodular amyloid.

DISCUSSION

Primary amyloidosis can occur anywhere along the urinary tract and has been reported in the kidney, renal pelvis, ureters, penis and even in the seminal vesicles (Gardner et al., 1971; Krane et al., 1973; Caldamone et al., 1980; Gulmi et al., 1988). While the etiology of amyloidosis is unknown, several theories suggest a chronic monoclonal inflammatory response or an immunologic mechanism (Cohen, 1967). Although bladder amyloidosis has been correctly diagnosed by cystoscopy in some reported cases, definitive diagnosis depends on histopathologic examination of the biopsy or resected specimen. Histologic examination shows proteinaceous amorphous eosinophilic deposits in the extracellular spaces. Diagnosis is confirmed by fluorescent apple-green birefringence after Congo red staining and visualization of the specimen under polarized light⁵. In a study conducted by Biewend et al, none of the twenty patients with primary localized amyloidosis developed systemic disease during the follow-up of 7.6 years (Biewend et al., 2006). This suggests that in primary

bladder amyloidosis, there is a low risk of progression to additional sites.

CONSLUSIONS

Primary amyloidosis of the urinary tract is a rare condition that mimics malignancy in its clinical presentation and cystoscopic appearance and diagnostic imaging. The physiopathology is unknown, the prognosis is usually good and there is no specific treatment. Early eradication with fulguration or transurethral resection is indicated. Cystoscopic follow-up is necessary. Literature recommends a long term follow up.

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**Nadir Rastlanan Xəstəlik: Ağrısız Hematuriyanın Səbəbi
Sidik Kəsəsinin İlkin Amiloidozudur**

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Sidik kəsəsinin primer amiloidozu nadir bir xəstəlikdir və ədəbiyyatda təxminən 200 belə xəstəlik bildirilmişdir. Klinik, sistoskopik və radioloji cəhətdən neoplastik xəstəlikləri təqlid edir. Bu xəstəliyin fiziopatologiyası bilinmir, proqnozu adətən yaxşıdır və bilinən bir spesifik müalicəsi yoxdur. Burada da biz nadir görülən sidik kəsəsi amiloidozu olan xəstəmizi, onun diaqnozunu və xəstəmizin müalicəsini təqdim etdik.

Açar sözlər: *Amiloidoz, transuretral rezeksiya, hematuriya*

**Редко Встречающаяся Болезнь: Причина Безболезненной
Гематурии – Первичный Амилоидоз Мочевого Пузыря**

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Первичный амилоидоз мочевого пузыря -это редкое заболевание, и в литературе отмечается примерно 200 таких случаев. Локализованный амилоидоз мочевого пузыря имитирует неоплазию клинически, цистоскопически и радиологически. Физиопатология этого заболевания неизвестна, прогноз, как правило, благоприятный, конкретного специфического лечения нет. В статье представлена информация о пациенте с первичным амилоидозом мочевого пузыря, а также данные о диагностике и лечении этого заболевания.

Ключевые слова: *Амилоидоз, трансуретральная резекция, гематурия*

ILHAM SHAHMURADOV – 60



Corresponding member of ANAS Ilham Ayyub Shahmuradov was born in 1958 in Gubadli district, Azerbaijan. In 1974 he graduated from Secondary School named by Gara Ilyasov in Gubadli settlement and entered the Applied Mathematics Faculty of the Azerbaijan State University. He graduated from the same faculty in 1979.

He began his research activity on the initiative of Professor Jalal Aliyev in the Biological Research Center, Azerbaijan National Academy of Sciences in 1979. He continued his studies at the Institute of Cytology and Genetics (IC&G) of the Siberian Division of the USSR Academy of Sciences (Novosibirsk), the largest and leading scientific center of the former Soviet Union in 1981-1982. I. Shahmuradov was PhD student at IC&G in 1982-1985 and he received his PhD degree in "Genetics" (supervised by Professor Vadim Ratner and Dr Nikolay Kolchanov) in 1987. He received his second scientific degree at the Institute of Botany of ANAS, Doctor of biological sciences in Genetics (scientific adviser: Professor J. Aliyev) in 2005.

In 1987-2016, I. Shahmuradov worked in various positions at the Institute of Botany, ANAS: researcher (1987-1989), senior researcher (1989-1992), Head of the Mathematical Modeling Group (1989-2000), leading researcher (1992-2002). He was head of the Bioinformatics Lab in the IB established on the initiative and with support of Professor J. Aliyev since 2002 until 2016. He has been a head of the Bioinformatics Lab at the Institute of Molecular Biology and Biotechnologies, ANAS since 2016. He was lecturer on molecular biology, genetics and bioinformatics at Baku State University,

Azerbaijan Medical University (AMU) and Khazar University in 1999-2015. He was professor at the AMU in 2012-2014.

In parallel with his activity in Azerbaijan, in 1999-2016, as an invited researcher, I. Shahmuradov worked at various research centers abroad: Sanger Center (UK, 1999), Helix Research Institute (Japan, 2000-2001), Royal Holloway College of London University (UK, 2001-2005), COMSATS Institute of Information Technology) at the COMSATS Information Technology Institute (Pakistan, 2007-2010) and King Abdullah University of Science and Technology (Saudi Arabia, 2015-2016).

The area of research interest of I. Shahmuradov is very wide, including structure and evolution of prokaryotic and eukaryotic genomes, organization and expression of genes in genomes; organelle-to-nucleus gene transfer in plants; development of bioinformatics tools for analysis of gene expression regulation mechanisms. A set of interesting results obtained by I. Shahmuradov together with colleagues from Russia, UK, USA, Pakistan and Saudi Arabia were published in peer-reviewed international journals, including the following ones:

- In 1980s, some structural evolutionary peculiarities of repeated DNA sequences of human, animal and plant species were revealed by the comparative computer analysis. It was suggested that these repeats covering a significant portion of eukaryotic genomes may participate in regulation of transcription of the neighbour genes/genome regions (Shahmuradov et al., 1986; Kapitonov et al., 1987). Later, this suggestion was confirmed by multiple experimental findings.
- I. Shahmuradov and his colleagues revealed that nuclear genomes of *Arabidopsis* and rice contain multiple plastid and/or mitochondrial DNA (ptDNA and mtDNA, respectively) splinters with intact copies of many organellar genes (Shahmuradov et al, 2003). Later, using the current annotations of nuclear genomes of these species, it was shown that nuclear copies of twelve known mitochondrial genes and five known plastid genes from *Arabidopsis* and rice, respectively, have known cDNA that supports occurrence of their transcription in nucleus. It was also revealed that the nuclear copies of eleven mitochondrial ORFs, annotated as hypothetical nuclear genes in *Arabidopsis*, are covered by long transcripts. PlantProm DB, a plant promoter database of annotated, non-redundant collection of the RNA polymerase II (Pol II) promoter sequences with experimentally determined transcription start site (TSS) from various plant species were developed (Shahmuradov and Solo-

vyev, 2003). The latest release of the PlantProm DB contains promoter sequences from 86 plant species (<http://www.softberry.com/plantprom2016/>). The database contains 576 entries including 150, 403 and 23 promoters of monocot, dicot and other plant genes, respectively.

- The studies of conserved features of regulatory regions of orthologous genes of mammals and plants revealed that major promoter functional components such as transcription start points, TATA-boxes and regulatory motifs, are significantly more conservative than the sequences around them (70–100% compared with 30–50%). To improve promoter identification accuracy in animal genes, these findings were employed in the computer program PromH (Shahmuradov and Solovyev, 2003).
- To predict Pol II promoters, the computer programs TSSP-TCM (Shahmuradov, Slovyev and Gammerman) and TSSPlant (Shahmuradov, Umarov and Solovyev, 2017) were developed. These tools predict both TATA and TATA-less promoters in sequences of a wide spectrum of plant genomes. Both computer programs achieved significantly higher accuracy compared to other available promoter prediction tools.
- The computational search for promoters in prokaryotes remains an attractive problem in bioinformatics. Despite the attention it has received for many years, the problem has not been addressed satisfactorily. In any bacterial genome, the transcription start site is chosen mostly by the sigma (σ) factor proteins, which controls the gene activation. The majority of published bacterial promoter prediction tools target σ 70 promoters in *Escherichia coli*. Moreover, no σ -specific classification of promoters is available for prokaryotes other than for *E. coli*. Recently, bTSSfinder, a novel tool that predicts putative promoters for five classes of σ factors in *Cyanobacteria* (σ A, σ C, σ H, σ G and σ F) and for five classes of sigma factors in *E. coli* (σ 70, σ 38, σ 32, σ 28 and σ 24) was developed (Shahmuradov et al., 2016).
- Nsite, NsiteM and NsiteH computer programs for discovery of motifs of transcription factor binding sites (TFBS) in animal and plant DNA sequences were developed (Shakhmuradov et al., 1986; Shahmuradov and Solovyev, 2015). Nsite performs search for all statistically non-random TFBS motifs. NsiteM searches for TFBS motifs estimated to be statistically non-random and also available in the all or a given portion of query sequences. NsiteH detects non-random and conservative TFBS motifs in the pair of orthologous genes.
- mRNA polyadenylation is an essential step of pre-mRNA processing in eukaryotes. Accurate prediction of the pre-mRNA 3'-end cleavage/polyade-

nylation sites is important for defining the gene boundaries and understanding gene expression mechanisms. I. Shahmuradov and colleagues developed a new computer program POLYAR for the prediction of poly (A) sites in human sequences (Akhtar et al., 2010).

I. Shahmuradov is an author of the first dictionary of bioinformatical terms in Azeri. He was elected a correspondent member of ANAS in 2014

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